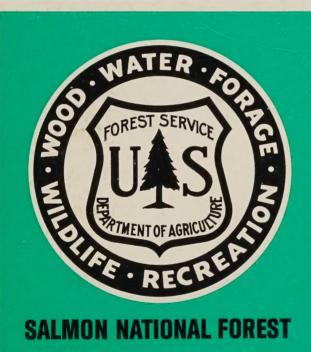
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MONITORING THE 1964 SPRUCE BUDWORM AERIAL SPRAY PROJECT



FOREST SERVICE
UNITED STATES DEPARTMENT OF
AGRICULTURE — OGDEN, UTAH

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MONITORING THE 1964 SPRUCE BUDWORM AERIAL SPRAY PROJECT



 $Prepared\ for$ Chief, Division of Timber Management by

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December 1965

FOREST SERVICE — UNITED STATES DEPARTMENT OF AGRICULTURE — OGDEN, UTAH

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FOREWORD

In recent years a serious infestation of spruce budworm developed in the timber stands of the Challis and Salmon National Forests in Idaho. The infestation intensified and magnified in area as the spruce budworm outgrew it natural enemies. As heavy losses in the 1.5 million acres of timber appeared certain, various methods to arrest the infestation were considered.

Satisfactory nonchemical measures to combat the spruce budworm have not yet been found.

After much deliberation and thorough investigation, Forest Service officials decided on aerial application of DDT as the best method of controlling a major part of the infestation. Concurrent with the decision to spray, administrative personnel were charged to take whatever steps were necessary to minimize the hazards to plants and animals associated with the infested timber as well as to decrease to a bare minimum the dangers to people. They also were instructed to carefully evaluate any impacts the control might have on forest environmental factors other than the target insect.

This report presents the method, measurement, and results of an intensive effort to monitor the impacts of large-scale insecticide spraying on various constituents of the forest community. Some phases of monitoring will extend into subsequent years. Supplemental reports will be issued as the results become available.

Much information in this report is directly attributable to the excellent cooperation and interest of other agencies and individuals, whose assignments were carried out with a high degree of professional ability. I take this opportunity to extend my sincere appreciation for their valued assistance.

Regional Forester

Floyd Therson

ACKNOWLEDGMENTS

Many individuals helped monitor this project. Their contributions of professional assistance are gratefully acknowledged. Particular recognition is due specific individuals who demonstrated personal interest and provided valuable assistance.

Joel Frykman, Chief, Timber Management Division, Intermountain Region, U.S. Forest Service, directed the overall project. He gave strong direction to the planning, conduct, and reporting of the monitor program. Paul Grossenbach, Branch Chief, Insect and Disease Control provided close personal guidance and assistance in conducting the monitoring and in editing this report.

Clifford Solberg, Project Leader, and Eugene Powers, Supervisor, Salmon National Forest, contributed generously to guidance in monitoring, all the way through final reporting. Charles Whitt, Fisheries Biologist, monitored the aquatic phases of the spray project. He contributed much personal time and energy to conducting his study and assembling data included in this report. George Gruell, Wildlife Biologist, competently and enthusiastically carried out his assignment to conduct the terrestrial portions of monitoring. Robert Patee, Project Air Operations Officer, made final analysis of all dye-cards used and compiled the portion of this report about comparison of the dye-card and filter-paper techniques. Many other Forest Service personnel displayed personal interest and assisted in many ways to monitor the project as completely as possible.

Idaho Fish and Game Department personnel were primary cooperators in the program. Director John Woodworth, Robert Salter, Assistant Director, and James Simpson, Chief, Fisheries Management, consulted with Forest Service personnel and directed activities of Department personnel. Fisheries Biologists, Ted Bjornn and Donald Corley, consulted with the Forest Service in programming all aquatic monitoring work. They conducted the special studies on Hughes Creek in 1963 and 1964. Their report, dealing

with these studies, is attached as an addendum to this report. A portion of the day-to-day stream monitoring for evaluating the effectiveness of protection measures was done by or under guidance of Mr. Corley. These same two men will report on the long-range effects of the DDT spraying on fish and aquatic insects at a later date.

Roger Williams, Big Game Supervisor, assisted in planning and conducting big-game sampling. Big-game collections in the Salmon area were under the supervision of Big Game Biologist Ralph Pehrson with assistance from local Conservation Officers, James Uranga, Lloyd Edwards, and Joseph Blackburn. Collections in Boise and Elmore counties were supervised by Big Game Biologist Richard Norell. Blue grouse collections were made by Bird Biologist Charles Blake.

Leland Fife, Dean Christensen, and Lee Eldridge of the Idaho State Department of Agriculture assisted in planning checks for agricultural products and in making collections for analyses. Darrel Brock and Cyril Maughan of the Idaho State Department of Health provided the same type of assistance for monitoring Grade A milk and culinary water. Joe Gabica did the analytical work for both of these state departments.

During the planning stages and field monitoring, Kenneth Walker, Agricultural Research Service, Yakima, Washington, was consulted on a number of occasions. He provided invaluable assistance in planning sampling techniques, and in acquainting project personnel with the technological aspects of residue analyses. He also expedited the residue analyses of the collections of birds, adipose tissues of big game, and all the water samples collected in aquatic monitoring. All filter paper field sample cards were furnished by and analyzed by the Agricultural Research Service Laboratory. Mr. Walker was transferred before the project was finished, and Lillian Butler supervised much of the analytical work done in Yakima. She maintained close liaison with Forest Service personnel

throughout the period of analyses and reporting. The Agricultural Research Service, through its personnel cooperating on this project, was a major contributor to the program evaluations.

Research Biologist Richard Pillmore of the Bureau of Sport Fisheries and Wildlife contributed invaluable assistance in planning terrestrial monitoring procedures. He assisted in the collection of prespray deer samples, of August deer samples, and of many of the vegetation samples. He gave additional assistance in preparing samples for shipment and analyses, and arranging for residue anlytical work at the Denver Research Laboratory.

Oliver Cope and Robert Bridges of the Bureau of Sport Fisheries and Wildlife assisted in planning the aquatic monitoring program.

SUMMARY

Since 1958 a spruce budworm infestation within the Salmon and Challis National Forests, Idaho, has spread and intensified. It increased to 1.5 million acres by 1963. More than 21 billion board feet of timber were threatened. Loss of the timber, mostly mature and over-mature Douglas-fir, could have caused a serious disruption in the area economy.

In July 1964, 525,000 acres within the Salmon National Forest, approximately one-third of the infestation, were treated with DDT to stop the serious losses which appeared imminent. Tributary drainages of the Salmon River from Horse Creek upstream to Carmen Creek below the town of Salmon, Idaho, were sprayed. Strips along fish producing waters, community water supplies, and other "sensitive" areas, where possible damage to other resources might result, were not sprayed. To maintain the best possible control of spray materials, approximately 41,000 acres adjacent to nonspray areas were sprayed by helicopters. The rate of application was 0.5 pounds DDT per acre. The remaining area was sprayed by fixed-wing craft dispensing 1 pound DDT per acre. Both application rates were in 1 gallon of diesel fuel per acre.

To protect against damage to other forest resources, additional provisions for control of spray materials were necessary. Observers in small planes and helicopters helped guide spray pilots and checked on proper control and application of spray materials. Pilots and observers were briefed daily. They also were given orientation flights to acquaint them with terrain, protection measures, and the best flight patterns. Spraying was terminated when weather conditions exceeded established safe tolerances for spray control. Homesteads, pastures, irrigation ditches, reservoirs, streams, nontimber types of more than 160 acres, and noninfested timber types were designated as nonspray zones.

This report presents findings of an intensive monitoring program which was conducted to evaluate forest environmental impacts re-

sulting from this spray project. Trained wild-life personnel of the Forest Service and Idaho Fish and Game Department were detailed to conduct fish and wildlife monitoring. Additional assistance was provided by other Federal agencies such as the Bureau of Sport Fisheries and Wildlife and the Agricultural Research Service. Other Idaho State agencies — the Department of Agriculture, and the Department of Health — also assisted.

Chinook and sockeye salmon, as well as steelhead trout, hatch and grow in or migrate through streams within the project area. The importance to the sport and commercial fisheries of these anadromous species as well as resident trout populations, necessitated adopting stringent measures for stream protection. Nonspray zones were established on each side of more than 700 miles of streams flowing five cubic feet or more per second. Helicopters applied a 0.5 pound DDT per acre in zones adjacent to nonspray areas.

Measuring abundance of drifting aquatic insects before and after spraying and using oilsensitive dye-cards to determine spray distribution were two methods used to check control effectiveness. As a result of surveillance, nonspray zones along the streams were widened twice during the project. Spray application by fixed-wing planes was moved back from the streams to twice the distance originally designed.

To determine the impact of spraying on aquatic insect fauna, bottom organisms were sampled before spraying, immediately after spraying, and again in October, 3 months later. In the few areas where excessive numbers of insects were caught in drift sampling, as an apparent result of spraying, little recovery occurred in bottom insect populations duringthe 3 months following spraying.

No streams studied suffered complete loss of bottom insects. Three of five streams sampled before spraying showed a decrease in total numbers in the first postspray evaluation. Two of these showed a marked recuperation by the October postspray sampling. Of 27

additional stations sampled immediately postspray, six did not show a marked increase in numbers by October. This situation cannot be identified as correlated with spray pollutants. Normal faunal dynamics of these streams are not known. There was a shift in the composition which was not entirely comparable to the change that occurred in the two control streams.

Insects of the orders Trichoptera (caddis flies) and Ephemeroptera (May flies) made up a smaller percentage in the fall samples than before and just after spraying. Seasonal fluctuations in aquatic insect numbers and composition are normal. Prior population measurments for the sample streams are lacking; thus, it is impossible to know what part of the measured changes may be natural and what part may be a result of spray materials getting in the streams.

Water samples were taken to help define possible correlations between aquatic insect losses and DDT in streams. Residue amounts in water were determined to a minimum detectable level of 0.2 part per billion. Only two samples indicated levels greater than one part per billion. Smaller amounts could in some cases be correlated with increases of aquatic insect losses, but not in all. Water samples taken continuously for periods of an hour or more apparently provided better evaluations of DDT in the stream than did periodic dip samples.

Chinook salmon and rainbow trout were held in live-boxes in streams within the project area and in control streams outside. Acute mortality of fish was not significantly higher in the project streams than in the control streams except where sudden drops in water levels appeared to be accountable for additional losses.

Many samples of various biological entities of the forest were taken. This was done to determine DDT residue levels that might result from the spray operation. In most cases, sampling was done before spraying, shortly after completion of spraying, and periodically for some time thereafter. Samples from control areas were also taken for comparative purposes. Cooperating agencies analyzed all samples.

Results from analyzing the various samples for total DDT and DDT metabolites are given in parts per million (ppm), without adjustments for recoverability or extractable lipid contents.

Two prespray samples of chinook salmon used in live-box tests registered 0.023 and 0.029 ppm. Two postspray samples showed 1.66 and 0.532 ppm.

All prespray samples of rainbow trout in live-box tests had detectable amounts from 0.054 to 0.234 ppm, averaging 0.115 ppm. In July the postspray sampling average rose to 1.608 ppm. In August the average was 1.687 ppm but it dropped to 0.328 ppm in October.

All wild fish sampled had measurable amounts of DDT residues in their body tissues. Prespray levels ranged from 0.020 to 0.410 ppm, averaging 0.117 ppm. In July, just after spraying was completed, the average rose to 0.875 ppm, and continued to rise to 1.037 ppm in October, 3 months after spraying.

Aquatic vegetation was sampled and analyzed. Six of 14 prespray samples had measurable amounts of DDT ranging up to 0.034 ppm. Only slight increases were noted in the October postspray samples, but each one did contain measurable amounts, ranging from 0.017 to 0.050 ppm.

Adipose (fatty) tissues were sampled from big game animals taken from within the project area before and after spraying and from animals well outside the project area as controls. Every animal from the project area had measurable amounts of DDT in the adipose tissue. Five prespray mule deer sampled had from 0.010 to 0.033 ppm. Nine deer sampled 1 month after the spray project ranged from 8.51 to 48.14 ppm, averaging 21.18 ppm. Two to 3 months after spraying 16 deer had an average of 19.36 ppm, ranging from 0.60 to 128.65 ppm. Thirteen elk taken 2 to 4 months after spraying ranged from 0.29 to 84.36 ppm, with an average residue level of 18.30 ppm. Five mountain goats a little over 1 month after spraying had residue levels ranging from 33.91 to 60.70 ppm, averaging 45.20. Samples of all three species taken from control areas ranged from less

than 0.01 ppm for most samples to a high of 0.25 ppm for a mule deer.

Rumen samples from four prespray mule deer samples had no detectable amounts of DDT. Samples from the rumen of five mule deer taken 1 month after the spray project varied from 27.0 to 185.3 ppm.

Whole-carcass analyses of robins taken as prespray samples from the project area showed levels from 0.26 to 3.13 ppm, averaging 1.165 ppm DDT. Postspray samples rose to an average of 3.16 ppm, ranging from 0.62 to 5.85 ppm.

Three blue grouse, taken after spraying had whole-carcass DDT levels of 7.84, 8.36, and 12.54 ppm.

Upland vegetation samples were taken from project sites known to have received DDT spray. This sampling was to evaluate the persistence of DDT on vegetation. Four species have been analyzed. There were no detectable levels in any prespray samples. Immediately after spraying levels ranged from 79.3 ppm for Douglas-fir to a high of 384.1 ppm for bluebunch wheatgrass. Levels in all species dropped abruptly within the next month, and 3 months after spraying levels were about one-half of what they had been immediately following spraying.

Samples from a number of agricultural products were analyzed. Cream samples from producers within and outside the project area showed no detectable levels of DDT before or after spraying. There were no Grade A milk

producers within the project area but samples from producers from 4 to 10 miles outside the project area had no detectable DDT levels. A sample of adipose tissue from one beef animal $4\frac{1}{2}$ months after coming off range within the project area, tested 5.19 ppm. Culinary water samples from public use sources had no detectable levels of DDT before or after spray operations.

This monitoring program was designed to measure residue levels but not to investigate the probable impacts from the sublethal levels which were found. No wild fish or warmblooded animals were found as acute losses during or after spraying.

A limited study was conducted of bird populations in two 40-acre plots. No marked shift in numbers was found between prespray and postspray censusing.

Under cooperation by agreement with the Forest Service, the Idaho Fish and Game Department is conducting a study on some long-range impacts of DDT on aquatic insects and fish. Results will be reported separately when the study is completed.

Under other agreements, the Idaho Fish and Game Department conducted special spray evaluation studies in 1963 and 1964. These were conducted on Hughes Creek, a stream within the 1964 project boundary. Special application patterns were made and a more intensive evaluation made of spray distribution and impacts on the stream. A report of these studies is attached to this report as an addendum.

THE AERIAL SPRAY PROJECT

PROJECT AREA

By 1963, approximately 1.5 million acres of timber on the Salmon and Challis National Forests in Idaho had become infested with spruce budworm (Choristoneura fumiferana Clem.). Douglas-fir (Pseudotsuga menziesii) was the primary timber type and carried most of the infestation. Engelmann spruce (Picea engelmanni) and subalpine fir (Abies lasiocarpa) made up small portions of the stands and were also infested.

In July 1964, 526,147 acres of the more heavily infested stands were sprayed with DDT. The project area (fig. 1) was entirely within the Salmon National Forest boundaries. It included infested timber types along the main Salmon River from about 15 miles north of Salmon, Idaho, down-river to and including Horse Creek and the main tributaries of Panther Creek and the North Fork of the Salmon River. Included also, as a small portion of the project area, was the upper end of the Camas Creek drainage, tributary of the Middle Fork of the Salmon River.

Terrain in the project area is extremely steep and rugged. Elevations range from 2,800 feet above sea level at the mouth of Horse Creek to several peaks above 8,000 feet. Most of the elevational changes are quite abrupt with 3,000- or 4,000-foot rises within 1 to 2 miles of the river. Access by roads is very limited and usually confined to the drainage bottoms. There is a network of several hundred miles of live streams in the project area, all of which are tributary to the Salmon River which flows westerly into the Snake River and on into the Columbia River.

OPERATIONS

Objective of the Spray Project

The purpose of this spray program was to reduce the population of spruce budworm to levels harmless to the timber stands, with a minimum of damage to other resources.

Plans

A multiple use survey report was made by rangers and the Salmon National Forest Supervisor. The basic responsibility for planning and operating the control project was assigned to the Chief, Division of Timber Management, and his organization in the Regional Office (Region 4) of the U.S. Forest Service. Coordination between project operations and the forest multiple use plan was maintained by close liaison between administrative project personnel and forest personnel.

The project was organized (fig. 2) to be handled directly by a designated Project Leader. Approximately 200 Forest Service personnel were assigned to the project. Many were detailed from the Regional Office, or the Salmon and other National Forests of the Intermountain Region. All others were hired in Salmon as temporary employees.

Individual plans prepared to facilitate the various phases of the project were for (1) operations, (2) entomology, (3) communications, (4) safety, (5) monitoring, and (6) information and education.

Spray Application

Two concentrations of DDT were used: 0.5 pound and 1 pound per acre. DDT powder was dissolved in an auxiliary solvent and formulated in fuel oil carrier. Applications of both mixtures were made at the rate of 1 gallon of fuel oil carrier per acre. The insecticide was furnished, mixed, and delivered to the planes by a contractor.

Five helicopters and 11 planes were used as spray craft on the project. All fixed-wing planes were converted military aircraft. There were eight TBM's, one B-25, one PB4Y2, and one C-47. Statistics on the accomplishments

Figure 1. The 1964 Spruce Budworm Project area boundaries. The project area is entirely within the Salmon National Forest.



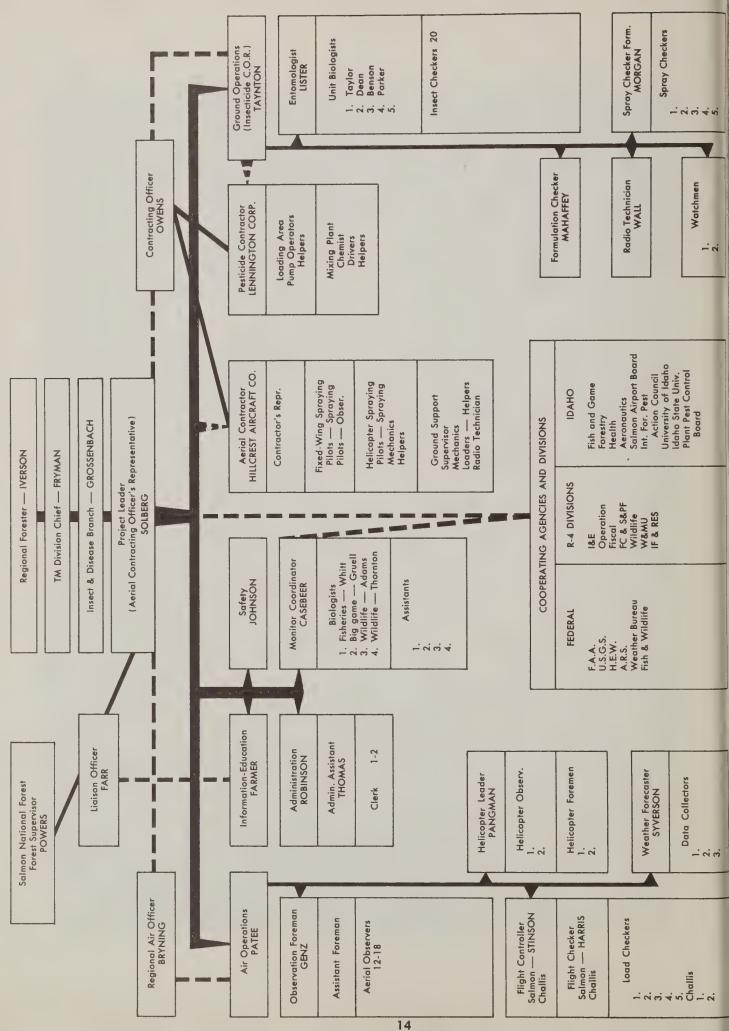


Table 1. Flight records by type of spray aircraft

Aircraft	Number of craft	Swath width (feet)	Spray capacity (gallons)	Total acreage sprayed	Flight time (hours)
Fixed-wing:					
TBM	8	400	750	422,815	401:04
B-25	1	250	1,000	36,789	26:38
PB4Y2	1	500	1,077	3,957	3:27
C-47	1	500	1,200	21,402	13:09
Subtotal	11			484,963	444:18
Helicopters	5	80	80	41,184	191:37
Total	16			526,147	635:55

of the various type of planes are shown in table 1.

Eleven planes (small 3-passenger) and three helicopters were assigned as observation craft. All planes and the spray helicopters were furnished and flown under contract with one prime contractor. Two observation helicopters were under a separate contract with the Forest Service and the third observer helicopter was Forest Service owned. Spray craft were paid for on an acreage basis and observation craft were paid for by the hour.

Helicopters started spraying July 2 and the first planes started on July 4. Helicopter application was finished July 16 and the planes finished July 24. It was necessary to coordinate spray application with spruce budworm larva development and feeding activity. Optimum budworm development occurred first at the lower elevations and progressed upwards, being closely associated with climatic conditions. On drainages with large elevational differences, it was necessary to first spray the lower portions and return later, possibly twice, to finish spraying higher elevations. This was often the situation for both helicopter and plane spraying. In many drainage basins spraying required several days.

Controls of Spray Application

No reports of similar aerial spray programs indicated such close control of insecticides as was provided in this project. Project organization personnel supervised operations and maintained and evaluated controls for the project. This assured operational safety, maintained efficient application of spray materials, and kept the impact on other environmental factors to a minimum, both in and out of the forest.

Experience gained on many aerial spray projects in recent years has resulted in development of specifications and operations which have improved the systematic and efficient application of insecticides. Controls are necessary to insure that spray materials are so applied that only the minimum amount needed for insect control will be used and will arrive on target and the least possible amount will fall "off-target," i.e., on other elements of the environment, where insecticide is neither needed nor wanted.

Close attention to, and enforcement of, control measures were instituted on this project. Evaluation of the effects of these controls and the impact of the insecticide on other environmental resources was conducted by the monitoring organization.

Spray aircraft are calibrated to release materials at given rates when flying at a predetermined speed, in horizontal flight, and within defined altitudinal ranges above the ground. The amount of spray reaching the target and

Figure 2. Organization and personnel of the 1964 Spruce Budworm Project.

how it is distributed is dependent also on climatic conditions. Contract terms specify tolerances in weather factors allowable for spray application. Only able and experienced pilots, flying in planes in top condition, can safely stay within all the established limitations throughout an extended project period.

Under conditions of extremely rugged terrain, as in the Salmon River country, it is practically a mechanical and physical impossibility to stay precisely within the established limitations at all times. A number of observations were made relating to the control of spray materials.

- 1. In rugged terrain, maximum elevations for spraying were often exceeded, especially by the larger multi-engine planes. This allowed a wider spread of spray on the ground than planned. It also allowed more influence from winds or localized thermal movements.
- 2. Either helicopters or planes will push spray materials out beyond swath boundaries when turning or changing craft altitude off the horizontal. This was of particular concern when spraying adjacent to sensitive areas in steep terrain. This problem was reviewed by project personnel with the contractor and pilots during the project.
- 3. Multi-engine planes used apparently were unable to maintain control of spray distribution as much as TBM's. Successful use of multi-engine type planes for DDT spraying will require additional study.
- 4. Daily briefing of pilots for both helicopter and fixed-wing planes included only occasional presentation of the monitoring program and results. Review of findings or some aspect of monitoring almost every day would probably help pilots to be constantly aware of this aspect of their job.
- 5. Orientation flights previous to spraying in any particular block were required of pilots and observers. Improved aerial photos or mosaics would provide better tools for acquainting all personnel more thoroughly with protection areas.

6. Specifications for weather limitations provided that spraying would be terminated when winds exceeded 6 miles per hour or when temperatures exceeded 68° F. Weather observation and forecasting were dependent on only three stationary stations located within the project area. Temporary stations were used occasionally when there was spraying in a particular area. Because of variable terrain and ground cover conditions, weather conditions often became erratic Localized situations often caused maximum conditions to be exceeded. These conditions no doubt contributed to occasional loss of control in spray distribution. Such conditions could not be detected without much more intensive coverage by weather observer stations.

A number of measures were incorporated in the operational plans to furnish special protection for various sensitive areas. Non-infested timber and open range areas larger than 160 acres, pastures, private lands, culinary water supplies and reservoirs, and strips around lakes and bordering streams carrying more than 5 cubic feet of water per second were designated as nonspray zones. Over 75,000 acres were thus delineated. Helicopters were used to spray only 0.5 pound of DDT per acre on strips bordering nonspray zones. All nonspray and helicopter spray zones were designated on project maps and on lap-boards used by pilots and observers.

Intensified use of lapboards and airborn observers was instituted in this project. Efficient application of these tools and techniques assisted greatly in controlling spray distribution.

Lapboards were aerial photo mosaics of individual block portions of the spray project area (fig. 3). Spray block boundaries and special zones to be protected were delineated on the mosaics. These lapboards served as ground control guides for all pilots and observers and were used for plotting daily spray accomplishments.

An observer in a small plane was assigned to each spray plane. Three helicopters, with an observer in each, were assigned to the five Figure 3. A typical lapboard mosaic. Boundaries of spray blocks and nonspray areas along designated streams were marked on each lapboard.

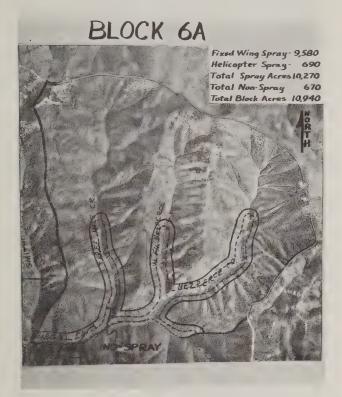


Figure 4. A spray helicopter. Five spray helicopters were under contract for the project. Each could carry a load of 80 gallons and was calibrated to spray a swath 80 feet wide. Helicopters were used for application where the most stringent control of spray was necessary. More than 41,000 acres were sprayed by helicopters in 191 flight hours.



spray helicoptors. All observers were Forest Service personnel. They helped guide spray pilots and were responsible for constant surveillance of the spray application to assure that planned techniques were followed.

Forest Service monitoring personnel flew with the helicopter observers 25 hours and with fixed-wing observers 24 hours. They evaluated the effectiveness of the planes, pilots, and observers in controlling spray deposit and in protecting the designated nonspray zones.

Radio communication between aircraft was essential to both spray pilots and observers. On occasions when radio contact failed, observer benefits were negated, and poor flight patterns or leaky spray nozzles could not be corrected promptly.

The Operational Report¹ for this project stated that observers should be highly qualified, experienced in map reading and aerial

¹Solberg, Clifford, Operational report, 1964 spruce budworm project, located on the Salmon National Forest, 1965. (Unpublished report on file at Intermountain Regional Office, U.S. Forest Service, Ogden, Utah.)



Figure 5. Eight converted military TBM planes flew a total of 401 hours and sprayed nearly 423,000 acres. Each plane could carry a load of 700 gallons of spray materials and was calibrated to spray a 400-foot swath.





Figure 6. One converted military PB4Y2 was used for about $3\frac{1}{2}$ hours before making an emergency crash landing. It was calibrated to spray a 500-foot swath and carry a capacity load of 1,077 gallons.





Figure 7. One converted military B-25 flew $26\frac{1}{2}$ hours and sprayed about 37,000 acres. It had a capacity load of 1,000 gallons and was calibrated to spray a swath 250 feet wide.





photo interpretation, have leadership abilities, and be capable of making quick decisions and taking independent action if needed.

Experience in monitoring this project varified the need for highly qualified personnel

as observers. Without qualified observers on the constant alert, spray pilots are subject to inherent pressures of completing a contract, and restrictive controls of spray distribution are easily minimized or neglected.



Figure 8. Observers were assigned to fly in separate helicopters and fixed-wing craft to guide and assist the spray pilot in maintaining the desired spray distribution pattern and protective measures. Monitor personnel accompanied helicopter observers for 25 hours and fixed-wing observers 24 hours for the purpose of evaluating observers and sprayplane performance.



Figure 9. Control of spray material is lost when it hangs too long in the air.



PROGRAMMING MONITORING

PURPOSE

For this project, monitoring was defined as "the measurement and determination of the impact of forest aerial spraying with DDT on components of the forest environment."

In recent years, the general public has become critical of the manner in which chemicals are being used to control man's environmental situations. Possible acute or immediate side effects from such uses have been noted. Recently, considerable attention has been focused upon the possible long-range effects which sublethal exposures to persistent insecticides may have on living organisms and components of their environment.

The spotlight of public concern is focused on pesticides because ever increasing quantities are being used each year. Although toxicological and pharmacological information is known for most commonly used pesticides, knowledge of the ultimate physiological changes which might result from their continued use is lacking. Added awareness of the presence of pesticides now exists because recently developed equipment and methods can detect residue levels of most pesticides to as little as one part per trillion, a much lower level than was formerly possible.

Passage of the Multiple Use and Sustained Yield Act of 1960, by Congress, formally charged the Forest Service with coordination of all facets of forest resources use, including the impacts of management activities on forest resources.

A multiple use survey report prepared on the Salmon National Forest prior to this aerial spray project defined needs for careful coordination of all elements of a spray project. Proposals by the Forest Service to the Federal Pesticide Review Board (now the Federal Committee on Pest Control) recognized the widespread public concern about the use of pesticides. The responsibility of resource managers to safeguard and evaluate the pesticide effects upon components of the forest environement, other than the target insect, also was noted.

OBJECTIVES

Objectives of the "Master Plan for the Monitoring Program for the 1964 Spruce Budworm Project," were to evaluate protective controls to verify their adequacy or modify the operations as needed, and to measure and analyze, insofar as possible, the impacts and effects of the DDT spray program on various components of the forest environment.

ORGANIZATION

Forest Service personnel from the Intermountain Region were detailed to the monitoring program. The Monitor Coordinator was responsible to the Project Leader (fig. 2). Four biologists served under the Monitor Coordinator (fig. 10).

Job descriptions were prepared for each type of position. Briefly stated, they were as follows:

- 1. Monitor Coordinator. He was responsible to the Project Leader for planning, coordinating, and administering the surveillance of all aerial spraying impacts upon the forest environment, except the spruce budworm.
- 2. Game Biologist. He assisted the Monitor Coordinator to plan, coordinate, and administer the terrestrial wildlife and wildlife habitat aspects of the monitoring program.
- 3. **Fishery Biologist.** He helped with planning, coordinating, and administering the fish and fish habitat aspects of the program.
- 4. **Biologist Assistant.** These two men assisted either the Game Biologist or Fishery Biologist in the conduct of field studies and the collection of biological specimens.

Figure 10. The monitoring organization for the 1964 Spruce Budworm Project.

1964 SPRUCE BUDWORM PROJECT

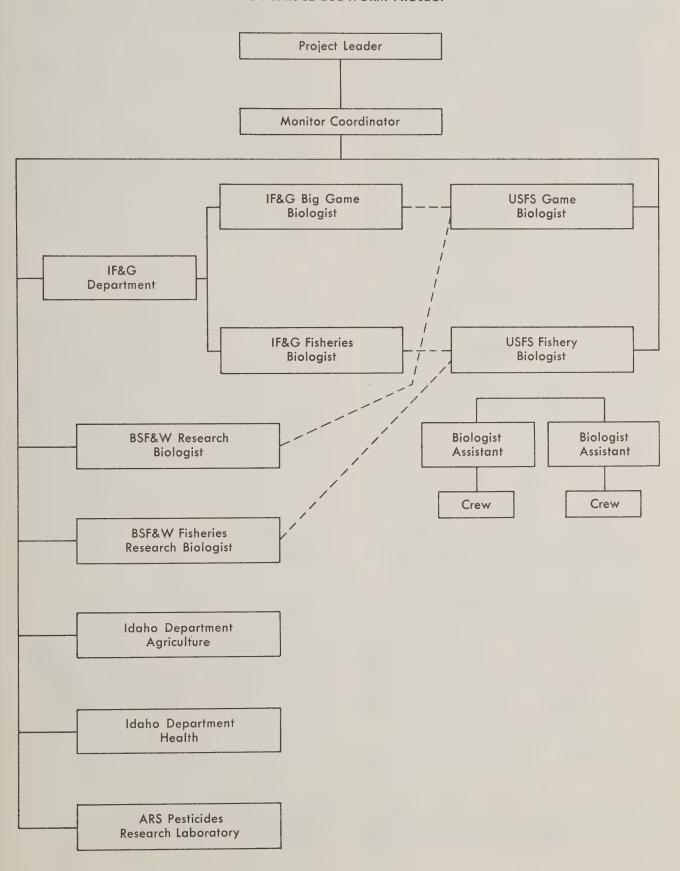


Table 2 lists detailers assigned and indicates some of their background and experience. Additional crew members were hired at Salmon on a temporary basis. During periods of extra heavy workloads in monitoring, personnel from other project activities were assigned to assist regular crews. This was done on a day-to-day basis when personnel were available.

Most preproject activity of the Monitor Coordinator was determining areas of responsibility, the availability of specialized services, and the solicitation of assistance from trained personnel in other governmental agencies. To assure that the monitoring program would be as complete as possible, many specialized services, such as pesticides residue analyses, were needed.

Many professional people displayed interest, a cooperative attitude, and participated willingly in this sensitive area of programing. This cooperative attitude did not necessarily imply approval of the spray project per se. Instead, it was a demonstration of willingness to assist in evaluating possible impacts of this project and to add scientific knowledge in the field of application and effects of insecticidal sprays.

Agencies taking active parts in the monitoring program were:

- (1) Idaho Fish and Game Department (on occasions referred to as IF&G),
- (2) Idaho Department of Health,
- (3) Idaho Department of Agriculture,

- (4) Bureau of Sport Fisheries and Wildlife of the United States Fish and Wildlife Service (on occasions referred to as BSF&W),
- (5) Entomology Research Division of the United States Agricultural Research Service (on occasions referred to as ARS).

The Monitor Coordinator made initial contacts with these agencies, solicited their participation, and drafted study plans for required approvals. He also coordinated agency studies and reporting of results. The relationship of cooperating agency personnel to project personnel is illustrated in figure 10.

AGREEMENTS

Forest Service representatives met with Idaho Fish and Game Department personnel April 8, 1964, and explained the proposed treatment program. A letter from Director Woodworth to Regional Forester Floyd Iverson on April 9 indicated no objections to the program as outlined. Mr. Woodworth said the Department would furnish assistance, but asked that the Forest Service finance this assistance.

Because of their basic responsibilities for fish and wildlife management, a cooperative agreement was negotiated between the Director and the Regional Forester whereby the Department would furnish technical assistance to carry out certain phases of aquatic

Table 2. Forest Service detailers assigned to monitoring program

Project		Dates on project		Detailing	Years in		ame Depart-
assignment	Name	From	То	National Forest	Forest Service		experience
		1964	1964	rorest	Service	Years	State
Monitor Coordinator	Robert Casebeer	4-23	7-241	Teton	6	12	Montana & Idaho
Fishery Biologist	Charles Whitt	4-23	8-5 ¹	Boise	5	7	Idaho
Game Biologist	George Gruell	5-11	7-24	Humboldt	2	5	California & Nevada
Biologist Asst.	Jack Adams	6-10	7-29	Cache	2	3	Utah
Biologist Asst.	Ivan Thornton	6-21	7-30	Dixie	4	_	_

¹These detailers spent time after this date on report writing.

and terrestrial monitoring. The Forest Service agreed to reimburse the Department for actual expenditures up to \$7,355.00.

Later as the spray operation progressed, there was mutual recognition that additional aspects of aquatic monitoring should be done. A supplemental agreement for the evaluation of some long-term effects from this spray program on acquatic insect populations and fish was, therefore, approved by the Director and the Regional Forester. The supplement provided for additional reimbursement up to \$3,007. There also was a mutual desire to study the impact of spray on forest grouse productivity. These arrangements involved no exchange of finances.

STUDY PLANS

In accordance with Forest Service instructions, an "Administrative Study Master Plan" was prepared for monitoring the 1964 Spruce Budworm Project and submitted to the Forest Service Regional Administrative Study Committee. This master plan was approved by the committee on May 13, 1964.

On April 29 a meeting of personnel interested in aquatic aspects of monitoring was held in Ogden, Utah. Representatives of the Denver Research Laboratory of the Bureau of Sport Fisheries and Wildlife, the Idaho Fish and Game Department, and the Forest Service attended. Arrangements were made for programing various phases of aquatic monitoring.

A similar meeting was held in Salmon, Idaho, on May 12 to program monitoring of terrestrial wildlife and habitat phases. Members of the above listed agencies and of the Agricultural Research Service attended. Arrangements were made as to how terrestrial studies should proceed.

Personal contact was made by the Monitor Coordinator with members of other agencies, and arrangements were made with each as to how they would assist.

Following these meetings and contacts, study plans were developed for individual phases of the monitoring program. These plans were reviewed by individuals scheduled to participate and were approved by representatives of each agency concerned.¹ Following is a listing and brief description of each of the study plans developed:

- 1. Monitoring terrestial wildlife. This plan provided for sampling big game: mule deer, elk, mountain goat (and possibly mountain sheep) by the Idaho Fish and Game Department; the collection of robins by the Forest Service; and the collection of vegetation by the Forest Service and the Bureau of Sport Fisheries and Wildlife. The robin samples and adipose tissues from big-game specimens would be analyzed for residues at the Agricultural Research Service Laboratory in Yakima, Washington. Other tissue samples from deer and the vegetation samples would be analyzed by the Denver Research Laboratory, Bureau of Sport Fisheries and Wildlife.
- 2. Monitoring fish and fish habitat. Responsibility for evaluating the impact of spraying on the aquatic areas within the project (except for Hughes Creek) was retained by the Forest Service. The plan provided for taking live wild fish samples and bottom aquatic insect samples before and after spraying; testing with chinook salmon and rainbow trout held in live boxes; sampling aquatic vegetation; and sampling water from streams. All samples would be analyzed at the Agricultural Research Service Laboratory. Day-to-day evaluation of the effectiveness of protective widths would be made by determining changes in numbers of drifting aquatic insects in the streams, by the use of dye-cards to measure spray distribution, and by monitoring personnel flying in observer craft to observe spray application. The Idaho Fish and Game Department was to provide assistance in evaluating the protective measures when personnel were available.

¹For individuals who are particularly interested, copies of study plans may be obtained from Division of Timber Management, U.S.F.S., Ogden, Utah.

- 3. Monitoring fish and fish habitat on Hughes Creek. A special test of protective widths was designed for Hughes Creek, to be evaluated by the Idaho Fish and Game Department as provided for in the cooperative agreement and the study plan. Originally, the plans specified leaving 100 feet of nonspray width on each side of streams, bordered by a 400-foot width for helicopter application of 0.5 pound of DDT per acre. The area beyond would be sprayed by planes at 1 pound DDT per acre. Later these plans were changed. A 400foot nonspray width was left on each side of the streams, bordered by a 400foot width of helicopter application of 1 pound DDT per acre, and the area beyond that was sprayed with 1 pound DDT per acre by TBM's. Forest Service personnel were to provide assistance in the evaluation. Samples collected would be analyzed at the Agricultural Research Service Laboratory.
- 4. Monitoring environmental factors other than fish and wildlife. A number of segments made up this plan:
 - a. Cream. Samples were scheduled to be taken before, and at designated periods after spraying. They were to be taken from producers both living inside the project and well outside the area. Collecting would be done by Idaho Department of Agriculture personnel and analyzed by their chemist in Boise, Idaho.
 - b. Grade A milk. Collections of samples were to be made before and periodically after spraying from producers living adjacent to and well outside the project area. Collecting would be done by Idaho Department of Health personnel and analyzed by their chemist in Boise, Idaho.
 - c. Beef cattle. A federal meat inspector of the Agricultural Research Service is on duty at the City Packing Com-

- pany, Salmon, Idaho. A list of forest permittees grazing cattle within the project area and another list of those grazing cattle well outside the project area were provided for the inspector. If cattle from either area were processed at the packing plant, adipose tissue samples would be taken and shipped to the Laboratory in Yakima for residue analyses.
- d. Culinary water. Water sources for public use were scheduled for sampling before and after spraying. Collections were to be made by Idaho Department of Health personnel with assistance from the Forest Service. Residue analyses would be conducted by the state chemist in Boise.
- 5. Long-term effects of this DDT project on aquatic insects and fish. The Idaho Fish and Game Department developed this plan in accordance with terms of a supplemental agreement between the Department and the Forest Service. For a 2-year period, Idaho Fish and Game Department personnel will do additional bottom sampling of aquatic insects and conduct additional exposure studies with brood fish, fingerlings, and eggs of chinook salmon, rainbow trout, and cutthroat trout. Tissue analyses were scheduled to be done at the Agricultural Research Service Laboratory.
- 6. Limited evaluation of the treatment program on blue grouse productivity. Idaho Fish and Game Department personnel planned to collect birds inside and outside of the project area in the fall of 1964 and again in the spring of 1965. Clutches of eggs were also to be taken in the spring for hatching at the Idaho Fish and Game Department bird farm in Jerome, Idaho. Observations of eggs and chicks were to be for hatchability, survival, condition, and DDT residue levels. The residue analyses work was scheduled to be done at the Agricultural Research Service Laboratory.

Table 3. Costs of Project and of Monitoring

Monitoring costs ¹		
Purchases	\$ 8,039.00	
Airplane travel	1,021.44	
Equipment rental	2,071.32	
Salaries	24,549.81	
Per-diem and travel	6,250.52	
Cooperative agreements		
Idaho Fish & Game Dept	10,362.00	
Total Monitoring Costs ²		\$ 52,294.09
Cost per acre		.099
Total costs of spray project		\$731,724.46
Cost per acre		1.39

¹Included in total costs of spray project. ²Forest Service costs. Contributions to monitoring by other agencies not included.

COSTS

All Forest Service costs for monitoring the project were paid from Insect and Disease Control funds allotted for the spruce budworm control project. Forest Service costs for the project and for monitoring are summarized in table 3. Only those funds paid to the Idaho Fish and Game Department under terms of agreements are shown here. Additional expenses for monitoring contributed by that Department and a number of other agencies are not included in the accounting shown in table 3.

DISCUSSION

Any one agency was not completely and properly equipped with personnel, knowledge, or equipment to properly assess the many ecological implications inherent in a treatment project of this nature. The Forest Service received much valuable assistance from a number of other federal and state agencies in conducting the evaluations of the possible impacts of this project. The willingness and ability of a number of interested professionals in the cooperative approach was demonstrated.

The monitoring organization was adequate for undertaking the program objectives. Original plans for monitoring terrestrial and agricultural phases were carried out very nearly

as planned. After starting the project, however, it became apparent that additional surveillance was necessary to more completely evaluate the effectiveness of control measures and the impacts of spray application upon the aquatic environment. Expansion required additional personnel as well as an additional workload on people already assigned to monitoring. These added workers were mostly other project assignees not engaged in their assigned duties at the time. This required coordination with other phases of the operations but resulted in more efficient use of detailers assigned to the overall project: Detailing of additional trained personnel to assume part of the workload could have been done, if this fact had been recognized early enough, and if qualified detailers had been available.

Additional workloads constituted some hazards to efficient monitoring. Without facilities to house and feed people out in the project area, it was necessary that project workers travel from 10 to 70 miles each direction over mountainous roads to do their jobs. Monitoring personnel had to be at their field stations at least an hour before daylight, the start of spray operations, and remain from 4 to 6 hours after spraying was finished. Sixteenhour days were common for all monitoring personnel. For short periods of a week to 10 days this type of schedule could have been

maintained with normal efficiency. But in this project 23 days, plus prespray field work, provided a schedule too strenuous for proper safety and best efficiency in monitoring. Portable field quarters, together with satisfactory communications with project headquarters, could have reduced the strain.

Experience in this surveillance indicated a need for earlier planning to avoid the possible shroud of an emergency or crash situation. Study plans need to be more detailed or refined to accomplish the desired efficiency in organization for the conduct of an intensive program of this nature.

It is generally recognized that aquatic environments must receive special protection during broadcast applications of persistent broad-spectrum insecticides such as DDT. Monitoring the protection measures of a large spray project such as this accomplished two major goals: (1) It provided a measure of the effectiveness of protection zones and an indication of the intensity of spray materials which might occasionally have gotten into the waters. (2) It established a persistent awareness on the part of all project and contract personnel that protection objectives were established with every intent of maintaining them throughout the term of the project.

MONITORING AQUATIC ENVIRONMENT

The Salmon River is well known for its populations of anadromous fishes. Each year thousands of chinook (Oncorhynchus tschawytscha) and sockeye salmon (Oncorhynchus nerka) as well as steelhead trout (Salmo gairdneri) ascend this river from the Pacific Ocean via the Columbia River to spawn. The progenies of these fishes remain in lakes, tributaries, and the main river for 1 to 3 years before returning to the ocean.

Salmon and steelhead trout which spawn in the Salmon River make up a valuable resource. Salmon River production of both species contributes greatly to the commercial fishery of the lower Columbia River. These fish are also highly prized for sport fishing which adds to the economy of communities located along the Salmon River and its tributaries.

During the freshwater phase of their life cycles, young anadromous fish depend primarily on aquatic insects for food. Many biologists believe that growth rates have much to do with survival to maturity. Thus, aquatic insects become extremely important with relation to growth, and, ultimately, the survival of these fish. Since aquatic insects are highly sensitive to DDT, and fish also are affected, this project warranted critical stream protection measures and surveillance.

EVALUATING STREAM PROTECTION MEASURES

Because of the extent and importance of the fisheries resource involved within this project, specific primary protection measures were instituted around streams and lakes to protect the fisheries resource. A major monitoring objective was to make frequent evaluations to determine adherence to both general and specific spray control specifications and the effectiveness of these measures on fisheries resource protection.

The original plan proposed for the 1964

project stipulated nonspray zones only 100 feet wide along each side of designated streams. Plans also provided that in steep terrain the nonspray area was to be widened to 200 feet when slope gradients adjacent to the stream ranged from 60 to 100 percent, and to 400 feet where slopes exceeded 100 percent. Beyond the nonspray zone a 400-foot strip was to be sprayed by helicopter applying 0.5 pound of DDT per acre. The remaining area was to be sprayed by fixed-wing craft applying 1 pound of DDT per acre.

At the April 29, 1964, meeting mentioned previously, Idaho Fish and Game Department and Forest Service biologists agreed on certain changes in the stream protection measures in view of findings in the 1963 Hughes Creek test project. They recommended that the nonspray zone be widened to 300 feet and that adjacent to the nonspray zone, a 400-foot swath be sprayed by helicopter applying 0.5 pound DDT per acre. The remaining area could then be sprayed at the rate of 1 pound of DDT per acre by planes.

Agreement was reached May 19, 1964, to start the project using these recommended stream protection measures with provisions for changing them during spraying if conditions warranted. Changes made during the course of the project are discussed later.

Previously, mutual agreement had been reached between personnel of the Idaho Fish and Game Department and the Forest Service, that all streams or portions of streams, estimated to be flowing 5 or more cubic feet per second (cfs) would be protected.

A preliminary designation of streams to receive protection was made by Forest Service rangers and Idaho Fish and Game Department fisheries biologists during the winter of 1963-64. An aerial survey was made in the spring to firm up these designations. Each stream within the spray area was observed. As a result of this survey, streams were added to the original list, making a total of about

Figure 11. Napias Creek runs approximately 60 cubic feet per second. More than 700 miles of streams running 5 cfs or more received special protection measures to protect fisheries values.



Figure 12. Aquatic insect drift sample locations were as follows: (1) Haynes Creek (control), (2) Freeman Creek, (3) Carmen Creek, (4) Sheep Creek, (5) Dahlonega Creek, (6) Nez Perce Creek, (7) West Fork Nez Perce Creek, (8) Anderson Creek, (9) Upper North Fork Salmon River, (10) Pierce Creek, (11) North Fork Salmon River (above Sheep Creek), (12) Hull Creek (above reservoir), (13) Hull Creek (at mouth), (14) Sage Creek, (15) Indian Creek, (16) West Fork Indian Creek, (17) Squaw Creek, (18) Spring Creek, (19) Boulder Creek, (20) Pine Creek, (21) Garden Creek, (22) Main Salmon River, (23) Colson Creek (24) Cramer Creek, (25) Corn Creek, (26) Wheat Creek, (27) Horse Creek (at mouth), (28) Stud Creek, (29) Horse Creek (above Stud Creek), (30) Horse Creek (above Saddle Creek), (31) Saddle Creek,

(32) Reynolds Creek, (33) Beaver Creek, (34) Phelan Creek, (35) Moccasin Creek, (36) Napias Creek, (37) Copper Creek, (38) Woodtick Creek, (39) Moyer Creek, (40) Musgrove Creek, (41) Porphyry Creek (east fork), (42) Porphyry Creek (west fork), (43) Porphyry Creek (at mouth), (44) Panther Creek (above Moyer Creek), (45) Panther Creek (above Musgrove Creek), (46) Panther Creek (above Porphyry Creek), (47) Panther Creek (above 4th of July Creek), (48) Panther Creek, (above Cabin Creek), (49) Cabin Creek, (50) Opal Creek, (51) Panther Creek (above Opal Creek), (52) Silver Creek, (53) Camas Creek (above Silver Creek), (54) Camas Creek (above Hammer Creek), (55) Little Jacket Creek, (56) Meadow Creek, (57) Beagle Creek, (58) Shovel Creek, (59) Yellowjacket Creek. —





560 miles. Portions of the Camas Creek drainage were included. These streams were designated on operational maps and delineated on lapboard mosaics.

A few days after the project had started. it was noted that a number of streams or portions of streams that had not been designated for protection measures were running at least the minimum of 5 cfs. Delayed spring runoff caused many streams to carry good flows of water when ordinarily they would have been dry, or practically dry, at project time. Thus, on July 14, additional stream mileages were added, making a total of more than 700 miles for the entire project area. Most additions resulted from extension of protection zones farther up the head waters of streams. or along additional tributaries. The additions were all in blocks which had not yet been sprayed at that date.

Aquatic Insect Drift Sampling

Aquatic insect drift sampling stations (fig. 12) were located a short distance above the mouth of each evaluation stream. Drift samplings began at 4:00 a.m. This was usually 30 minutes to 1 hour before spraying began. Samples were collected hourly until 4:00 p.m. Samples taken before spraying started are classed as prespray samples while those collected after spraying began are classed as postspray samples.

The collection site on each stream was located and marked so successive samples could be taken at the same site. Locations were selected, when possible, where water was 11 to 12 inches deep. This depth allowed the 12-inch-square openings of the net to sample from the bottom, sub-surface, and surface of the stream. The sites were also located near the centers of the main stream currents. Slower waters were sampled when possible to prevent the smaller insects from being mangled or swept through the netting.

The sampling apparatus consisted of a wire screen, bag type sampler with an opening 12-inches square. The bag was constructed from aluminum 144 mesh (openings per square inch) screening.

Forest Service biologists sampled for 5-minute periods which had been indicated by Graham and Scott¹ as adequate. An exception was at the station on the main Salmon River. Idaho Fish and Game Department biologists sampled for 5-, 10-, or 15-minute periods, depending on the numbers of insects in the samples taken prior to spraying.

Insects collected in each sample were placed in individual vials of alcohol. Samples were identified by numbers on vial lids and on pieces of paper placed inside the vials. Collection information was recorded on field record forms (fig. 14).

Under normal conditions some insects can be found drifting downstream at all times. The number of insects collected prior to a spray operation is the prespray number or level, and the number collected during or after spraying is called the postspray number or level.

Bridges and Andrews (1961) found that a dosage of .002 parts per million DDT was sufficient to make aquatic insects lose their hold on rocks. Insects collected in drift nets were not always dead at time of collection. Most insects collected, however, were either moribund or in an excited state.² Jensen and Gaufin (1964a) in reporting their work with two species of stonefly naiads stated that when the insects exhibited tremors and convulsions, death usually occurred within a few hours. Scott and Kucera² go on to say that such insects show the first symptoms of a nerve poison, and death is quite certain.

Numbers of aquatic insects collected in prespray drift collections vary widely between samples. Variations of several hundred per-

¹Graham, R. J. and Scott, D. O., Effects of an aerial application of DDT on fish and aquatic insects in Montana, 1959. (Joint project report of Montana Fish and Game Department and U.S. Forest Service, on file with Montana Fish and Game Department, Helena, Montana.)

²Scott, D. O. and Kucera, D. R., Effects of DDT and malathion spraying on aquatic insects in the Bitterroot River drainage, 1964. (Unpublished report on file with Division of State and Private Forestry, Region 1, U. S. Forest Service, Missoula, Montana.)

cent between samples are normal. On the other hand, it is recognized that minute amounts of spray falling into a stream can result in an increase of drifting insects which may amount to less than that which may occur by normal variation. Thus, it is difficult to evaluate whether small variations between prespray and postspray levels are normal variations or due to spray application.

Prior to the start of the Salmon National Forest spray project, Forest Service and Idaho Fish and Game Department biologists agreed that an increase of 400 percent or more between prespray and postspray levels would be construed as meaning the increase was probably due to insecticide.

The formula used for computing drift sample increases was as follows:

Average of three highest consecutive postspray samples — Average of prespray samples

(Average of prespray samples)

X100 = Percent increase

A listing of aquatic insect drift sampling data is shown in table 4.

Figure 14. Aquatic insect drift and bottom sample field record form.

	Sample Co	ollector	
Vial Sample No.			
Date			
Station Name or Number			
Time			
Length of Time			
Sampling Method			

Organism	No.	Vol.										
1												
2												
3												
4												
5												
6												

Table 4. Detailed listing of aquatic insect drift sampling

Protection width				Date		Samplina		Driffing aquatic Insects	Secto
(feet)	(pounds per acre)	Type of aircraft	Name of stream	sampled (1964)	Sampled by	period (minutes)	Prespray	Postspray	Percent increase
Prespray			Pine	7-1	USFS	5	9.4		
"			Spring	7-2	USFS	5	3.8		
"			Beaver	7-3	IF&G	5	5.2		
"			Dahlonega	7-6	USFS	5	3.9		
"			Opal	7-12	USFS	5	13.7		
							Avg. 7.2 ²		
0	z.	'Copter	Porphyry #2	7-15	USFS	5			
0		'Copter		7-15	USFS	5	3.5	270.0	7,614
0	1.0	PB4Y2		7-16	USFS	5	3.5	34.3	880
300	5:	'Copter	Boulder	7-2	IF&G	5	3.6	36.7	919
"	"	*	Horse	7-2	IF&G	15			
*	"	"	Pine	7-2	USFS	5			
"	=		Corn	7-3	USFS	5			
"	"	"	Garden	7-3	IF&G	5			
"	"	×	Spring	7-3	USFS	5			
"	"		Wheat	7-3	USFS	5			
	*	"	Colson	7-4	USFS	5			
*	=		Garden	7-4	USFS	5			
	"	z	Indian	7-4	USFS	5	5.4	50.3	831
=	×	"	Squaw	7-4	USFS	5			
400	.c.	'Copter	Hull	7-6	USFS	5			
"	"		N. Fk. Salmon	7-6	IF&G	15			
"	"		Sheep	7-6	IF&G	15			
"	2	"	Anderson	7-10	USFS	5			
900	٤.	'Copter	Opal	7-15	USFS	5			
"	"	"	Porphyry #1	7-15	USFS	5			
"	"	*	Porphyry #6	7-15	USFS	5			
"	z.	"	Porphyry #7	7-15	USFS	5			
800	1.0	TBM	Garden	7-6	USFS	5			
,,	"	"	Cramer	7-7	IF&G	15			
*		"	Indian	7-7	IF&G	15			

Table 4. (continued)

		1		Date		Sampling	Driffi	Drifting aquatic insects ¹	nsects
Protection width (feet)	DDI applied Type of (pounds per acre) aircraft	Type of aircraft	Name of stream	sampled (1964)	Sampled by	period (minutes)	Prespray	Postspray	Percent increase
800	1.0	TBM	Pine	7-7	USFS	5			
"	"	"	Pine	7-8	USFS	5	9.4	122.7	1,205
"	u	"	Indian	6-7	IF&G	15			
"	n n	"	Boulder	7-10	IF&G	15	3.6	26.0	622
"	n n	"	Spring	7-10	USFS	5	3.8	51.0	1,242
"	II	"	Spring	7-10	IF&G	15	3.8	61.7	1,524
"	"	"	Horse	7-12	IF&G	15	5.0	65.3	1,206
*	*	*	Dahlonega	7-12	IF&G	15	3.6	436.6	12,028
800	1.0	B-25	Woodfick	7-11	IF&G	15			
"	*	"	Opal	7-13	USFS	5			
1200	1.0	TBM	Carmen	7-14	USFS	5	7.7	237.3	2,982
1200	1.0	B-25	Woodtick	7-14	IF&G	15	6.2	186.6	2,910
1400	1.0	TBM	Napias	7-15	IF&G	15			
"	"	"	Moccasin	7-15	IF&G	15	7.23	152.5	2,018
"	"	"	N. Fk. Salmon	7-16	USFS	5			
"	"	"	Twin	7-16	USFS	5			
"	"	"	Twin	7-17	USFS	5			
"	"	"	N. Fk. Salmon	7-17	USFS	5		•.	
"	"	"	Camas #1	7-18	IF&G	15			
*	"	"	Camas #2	7-18	IF&G	15			
"	"	"	Panther-4th						
			of July	7-18	IF&G	15			
"	"	*	Musgrove	7-19	IF&G	15			
*		"	Panther-						
			Porphyry	7-19	IF&G	15			
"	*	*	Panther-						
			Musgrove	7-19	IF&G	15			
"	"	*	Panther-Cabin	7-19	IF&G	15			
"	*	*	Porphyry	7-19	IF&G	15			
"	"	*	Cabin	7-19	IF&G	15			
"	"	*	Shovel	7-19	USFS	5	7.23	63.3	779

Table 4. (continued)

		1		Date		Sampling	Drittin	Drifting aquatic insects.	sects.
Protection width (feet)	DDT applied Type of (pounds per acre) aircraft	Type of aircraft	Name of stream	sampled (1964)	Sampled by	period (minutes)	Prespray	Postspray mean	Percent increase
1400	1.0	TBM	Shovel	7-20	USFS	5			
"	"	"	Anderson	7-21	USFS	5			
"	"	"	Horse #1	7-21	IF&G	15			
"	"	*	Horse #2	7-21	IF&G	15			
"	"	"	Nez Perce #1	7-21	IF&G	15			
"	"	"	Nez Perce #2	7-21	IF&G	15			
"	n n	"	Reynolds	7-21	IF&G	15	4.8	55.3	1,052
"	"	"	Saddle	7-21	IF&G	15	5.0	267.0	5,240
"	"	"	Stud	7-21	IF&G	15			
"	"	"	Dahlonega	7-21	USFS	5			
"	"	"	Carmen	7-22	USFS	5			
"	"	"	Anderson	7-22	USFS	5			
"	"	"	Napias	7-22	IF&G	15			
"	"	"	Phelan	7-22	IF&G	15			
"	"	"	Phelan	7-23	IF&G	15			
"	"	"	Napias	7-23	IF&G	15			
"	"	"	Freeman	7-23	IF&G	15			
"	*	=	Carmen	7-23	IF&G	15			
1400	1.0	B-25	Copper	7-15	IF&G	10	7.23	64.7	800
"	"	n n	Moyer	7-15	IF&G	15			
"	"	"	Silver	7-15	IF&G	15	5.0	237.3	4,646
"	"	"	Silver	7-18	IF&G	15			
n n	"	"	Opal	7-22	USFS	5			
"	"	"	Panther-Opal	7-22	USFS	5			
"	"	"	Opal	7-23	USFS	5			
"	"	"	Panther-Opal	7-23	USFS	5	3.0	36.3	1,110
"	"	PB4Y2	Musgrove	7-15	IF&G	15			
n	"	*	Porphyry #7	7-15	USFS	5			
"		"	Porphyry #1	7-16	USFS	5			
"	"	"	Porphyry #6	7-16	USFS	5			
"	"	"	Porphyry #7	7-16	USFS	5			

Table 4. (continued)

				Date		Sampling	Driftii	Drifting aquatic insects ¹	sects ¹
Protection width (feet)	(pounds per acre)	Type of aircraft	Name of stream	sampled (1964)	Sampled by	period (minutes)	Prespray	Prespray Postspray mean mean	Percent increase
1400	1.0	C-47	Beagle	7-19	USFS	5	7.23	45.7	530
"	"	"	Meadow	7-19	USFS	5			
"	"		Beagle	7-20	USFS	5			
"	"	"	Little Jacket	7-20	IF&G	15			
"	"	ž.	Meadow	7-20	USFS	5			
Combinations:4									
300 & 700	.5 & 1.0	Hel. & TBM	Beaver	7-4	IF&G	15	5.2	38.0	631
400 & 800	.5 & 1.0	Hel. & TBM	Sage	7-8	IF&G	15	14.6	586.6	3,918
400 & 800	.5 & 1.0	Hel. & TBM	Dahlonega	7-10	USFS	5	3.6	19.7	447
400 & 800	.5 & 1.0	Hel. & TBM	N. Fk. Salmon	7-11	USFS	5	7.4	756.3	10,120
400 & 800	.5 & 1.0	Hel. & TBM	Pierce	7-11	IF&G	15	7.23	1,000.0	13,790
400 & 800	.5 & 1.0	Hel. & TBM	N. Fk. Salmon	7-13	USFS	5	7.4	94.3	1,174
400 & 1200	.5 & 1.0	Hel. & B-25	Panther-Moyer	7-14	IF&G	15		•	
400 & 1200	.5 & 1.0	Hel. & B-25	Moyer	7-14	IF&G	15			
600 to 1000	5.	Hel.	Holl	7-8	USFS	5			
1400	1.0	C-47 & TBM	Yellow Jacket	7-19	USFS	5	7.2	108.0	1,400
1400	1.0	C-47 & TBM	Yellow Jacket	7-20	USFS	5	7.23	44.0	511

¹Where no figures are shown there was less than 400 percent increase from the prespray mean to the postspray mean.

²Average of 5 prespray days.

³Prespray average based on ².

⁴Two types of aircraft spraying in same drainage on the same date.

⁵Estimated mean.

Ninety-six stations were operated for obtaining data on streams where spraying was modified. This disregards the prespray samples and the four for Porphyry Creek, where no stream protection measures were applied. Approximately one-half the stations were manned by Forest Service personnel and the other half by Idaho Fish and Game Department personnel.

Station statistics are summarized in table 5 according to percent of hits by type of aircraft and minimum spray distance from the stream.

As shown in table 4, many streams were monitored on several occasions — different days and sometimes for different types of aircraft. In all, monitoring was conducted on 44 streams. On three of these, however, there was more than one station. There were stations at six locations on Panther Creek. Porphyry had three stations, and, although not indicated in table 4, there were at least two stations on the North Fork of the Salmon. Therefore, table 6 summarizes "by streams"

which means individual streams as they were flown by one type of aircraft for one protection width. Some streams are included in more than one category, thus indicating a total of 67 streams instead of 44.

In addition to using the 400-percent increase level as a basis for evaluating stream protection specifications, both agencies agreed to consider other possible extenuating circumstances before making recommendations for changes in operational procedures. It was necessary to ascertain if spray application complied with operational specifications. The possibility existed that changing weather could adversely affect spraying before operations could be stopped. Also, improper flight patterns and/or faulty equipment could cause undesirable impacts. But these problems could be corrected without need for changes in protection widths. It was agreed, therefore, that professional judgment would be exercised in determining if excessive increases in drifting insects were due to operational procedures or to inadequate stream protection specifications.

Table 5. Summary of insect drift sampling results (by stations) as related to type of aircraft and stream protection zone widths

Type of	Minimum distance	Stations havi 400 percei		Stations having 400 perce	g greater thar nt increase
aircraft	from streams -	Number	Percent	Number	Percent
Helicopter	300	9	82	2	18
Helicopter	400	4	100	0	0
Helicopter	600	5	100	0	0
TBM	800	5	45	6	55
TBM	1200	0	0	1	100
TBM	1400	30	88	4	12
B-25	800	2	100	0	0
B-25	1200	0	0	1	100
B-25	1400	5	62	3	38
C-47	1400	4	80	1	20
PB4Y2	1400	4	100	0	0
C-47 & TBM1	1400	1	50	1	50
Hel. & TBM1	300-700	0	0	1	100
Hel, & TBM1	400-800	0	0	5	100
Hel. & B-25 ¹	400-1200	2	100	0	0
Totals		71		25	

¹Both types of aircraft operated in the same drainage on the same day.

Table 6. Summary of insect drift sampling results (by streams) as related to type of aircraft and stream protection zone widths

Type of aircraft	Minimum distance from streams	Streams havin 400 perce		Streams having 400 perce	g greater than nt increase
	rrom streams	Number	Percent	Number	Percent
Helicopter	300	8	80	2	20
Helicopter	400	4	100	0	0
Helicopter	600	3	100	0	0
TBM	800	3	38	5	62
TBM	1200	0	0	1	100
TBM	1400	16	80	4	20
B-25	800	2	100	0	0
B-25	1200	0	0	1	100
B-25	1400	2	40	3	60
C-47	1400	2	67	1	33
PB4Y2	1400	2	100	0	0
C-47 & TBM1	1400	0	0	1	100
Hel. & TBM ¹	300-700	0	0	1	100
Hel. & TBM ¹	400-800	0	0	4	100
Hel. & B-25 ¹	400-1200	2	100	0	0
Totals		44		23	

¹Both types of aircraft operated in the same drainage on the same day.

To assist in interpretations of findings, monitoring personnel flew in observer craft, both fixed-wing and helicopters, to observe spray operations from the air.

Salmon River Station

Drift-sample data, shown in table 7, collected at the main Salmon River station, were not incorporated with other drift-sample data because of differences in collection techniques. Drift samples at this station were obtained by sampling with the drift net just below the surface in water approximately 4 feet deep. Also, the location was only 10 feet from shore, which was 25 to 30 feet from the center of the main current. A 10-minute sample was taken each hour for 10 consecutive days.

Data from table 7 are shown graphically in figure 15 where the number of insects collected were averaged by hourly periods. These data show the cyclic effects of nocturnal activity. Nocturnal activity stems from normal motivations related to changing light conditions (Waters, 1962).

The small peak occurring at 9:00 a.m., in all probability resulted from upstream spraying activity.

Oil-sensitive Dye-cards

Oil-sensitive dye-cards were located at strategic points along streams to measure amounts of spray reaching the ground and its distribution with relation to streams. Because of manpower and time restrictions, only minimum card locations were employed. The three following patterns were used:

Major transects. Major card-line transects were designed by placing card series at right angles to the stream. Cards were spaced 50 feet apart (slope distance), starting at the edge of the water, for a total distance of 700 feet (horizontal distance) on each side of the stream. Transects on relatively flat ground, therefore, contained 15 cards on each side of the stream. Transects on steeper slopes contained as many as 18 cards on each side of the stream. Each transact also contained one card placed 1 to 3 feet above the center of the stream.

Table 7. Summary of drifting aquatic insect samples collected at the main Salmon River station (Number of aquatic insects collected per 10-minute drift sample)

Date						Time (a.m.)					
(1964)	12:00	1:00	2:00	3:00	4:00	5:00	6:00	7:00	8:00	9:00	10:00	11:00
7/6												
7/7	13	6	15	14	5	4	3	3	1	5	19	35
7/8	16	12	11	19	4	1	1	3	2	3	7	5
7/9	5	17	12	8	2	0	6	1	2	2	0	1
7/10	6	9	12	7	6	0	3	1	3	2	1	0
7/11	7	7	9	14	4	1	2	2	1	4	1	0
7/12	3	3	3	4	0	1	0	2	0	4	1	0
7/13	6	7	13	4	1	1	3	3	2	3	3	1
7/14	14	15	10	11	2	5	1	2	2	1	1	1
7/15	15	16	16	10	6	3	2	3	2	0	0	0
7/16	11	11	17	12	7	2	1	0	0	0		
Total:	96	103	118	103	37	18	22	20	15	24	33	43
Avg.:	9.6	10.3	11.8	10.3	3.7	1.8	2.2	2.0	1.5	2.4	3.7	4.8
Date						Time	(p.m.)					
(1964)	12:00	1:00	2:00	3:00	4:00	5:00	6:00	7:00	8:00	9:00	10:00	11:00
7/6							5	3	3	14	25	22
7/7	7	0	11	3	4	6	4	1	6	11	24	24
7/8	4	1	1	2	3	1	2	3	5	4	15	7
7/9	1	0	1	1	0	0	0	1	3	5	25	19
7/10	0	3	1	2	1	1	4	2	2	6	19	8
7/11	1	0	1	0	0	1	1	2	1	5	12	15
7/12	0	0	1	1	0	0	0	1	5	5	16	9
7/13	0	0	0	0	0	2	1	2	1	2	10	12
7/14	1	1	1	2	2	2	1	0	1	5	7	10
7/15	1	2	0	0	1	4	0	0	2	5	14	4
7/16												
Total:	15	7	17	11	11	17	18	15	29	62	167	130
Avg.:	1.7	.8	1.9	1.2	1.2	1.9	1.8	1.5	2.9	6.2	16.7	13.0

Small benches were dug in the hillside, when necessary, to place the cards in a level position. A nail pushed through the card into the ground held each card in place. A code-numbering system identified each card and recorded the name of the stream, date, number of the transect, and position of the card on the transect line.

Minor transects. Minor transects were similar to major transects except for length. These transects extended only 100 feet (horizontal distance) on each side of the stream. Most minor transects contained a

total of three cards on each side plus one above the center of the stream. Minor transects were located between major transect lines.

Lateral transects. A lateral transect was a line of cards placed along a road parallel, or nearly parallel, to a stream. In most cases the axes of lateral transects varied from 50 to 200 feet from the stream. Card spacing varied from 200 feet to 1/5 of a mile.

Dye-cards were quantitatively evaluated by visual comparison with a series of standard

Figure 15. The average of 10-minute drift samples collected during the same hour for a 10-day period, Salmon River Station.

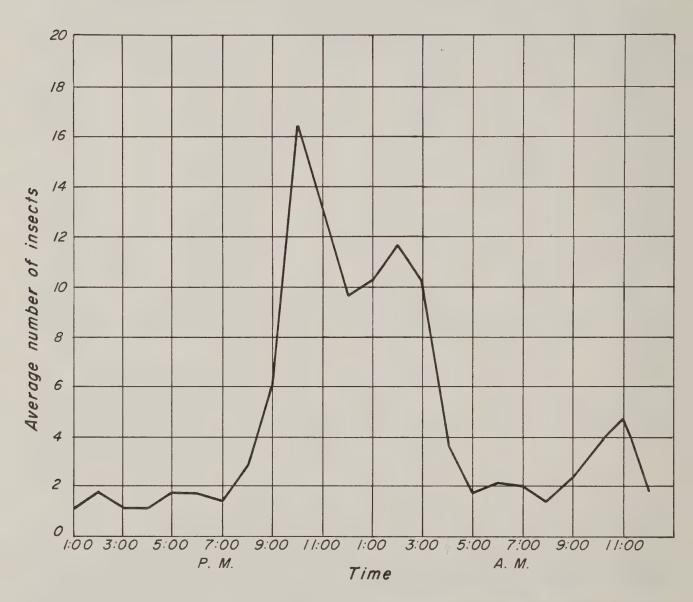


Figure 16. Oil-sensitive dye-cards were used to determine spray distribution reaching ground level. Used in monitoring to evaluate spray distribution adjacent to streams. Card on left has not received any spray materials. That on the right shows response of emulsion to oil used as carrier for insecticide.



index cards. Dye-cards are oil sensitive and actually indicate the amount of oil in gallons per acre. Index cards are calibrated to represent spray concentrations ranging from .01 to 4.4 gallons of oil per acre. Interpretation for DDT amount was based on the formulated mixture of DDT per gallon of oil.

In general, dye-card evaluations indicated a close correlation between the spray distribution pattern and aquatic insect mortality.

Dye-card transects were used in monitoring 10 different streams. Along four of these streams the dye-cards were used on two different spray days and one stream was checked for three days. Of the 16 sampling days, 6 had indications of measurable amounts (.005 to 0.2 pound DDT per acre) of spray within 200 feet of the stream. Each of these six instances was concurrent with more than a 400 percent increase in drifting aquatic insects in the stream.

Evaluations of spray cards also revealed that helicopter pilots tended to be conservative in flying their spray patterns during the first days of the project; i.e., the spray pattern was usually located a greater distance from the stream than was planned. Examples of this conservatism were revealed by dyecards. When helicopters were supposed to apply spray 300 feet from the stream, pilots flew Pine Creek an average minimum distance of 600 feet from the stream and on Spring Creek, the distance ranged from 250 to more than 700 feet. On the east side of Colson Creek only traces occurred on cards located 700 feet from the stream while no cards on the west side showed spray droplets.

Plane pilots generally flew at conservative distances from the streams according to observations from the ground and air. Drifting spray in concentations of .01 pound or more per acre was found, however, on cards within a few feet of several streams. On Carmen Creek, ground observers reported the TBM pilot apparently flew a good application pattern. Traces, however, were found on cards located ³/₄ of a mile from the spray block. In this case, the ground observer measured erratic wind gusts up to 10 miles per hour during spraying.

A special study was conducted on the use and evaluation of dye-cards in comparison with another technique. This is reported later under "Special Situations."

Stream Protection Changes

Spraying operations began July 2, 1964. Only three streams were to be monitored during the first 3 days of operation. A total of 13 streams, however, were monitored, with assistance of Idaho Fish and Game Department personnel and with additional personnel assigned to Forest Service monitoring crews.

During the first 2 days of spraying adverse results were noted in only one of eight streams monitored. Boulder Creek showed an increase of approximately 920 percent in drift of aquatic insects (table 4). As discussed previously, oil-sensitive dye-cards indicated that helicopter spray pilots were being extremely conservative in their spraying patterns.

The question had not yet been answered as to whether or not it was possible to get spray application at the specified distances from the stream so as to attain spruce budworm control and still maintain adequate stream protection. Therefore, on the third day a concerted effort was made to spray the helicopter zone of a selected drainage according to the specifications, i.e., from 300 to 700 feet from the stream. Close control was maintained in spraying Indian Creek in an effort to place the spray down within that band.

Results showed an aquatic insect drift of about 830 percent increase over the prespray level. Dye-cards along a lateral transect showed concentrations of 0.015 pound of DDT per acre commonly occurred between 100 and 250 feet from the stream. Some cards located on bridges and within 50 feet of the streambanks showed concentrations of 0.005 pound of DDT per acre.

At the same time Indian Creek was being sprayed, Colson Creek was also being treated but without the concerted effort to adhere to specifications. On Colson Creek dye-card transects showed no spray within 700 feet of

the stream, and no aquatic insect losses occurred. Three other streams monitored on the third day of operations showed less than the 400 percent increase in aquatic insect drift.

On July 5, adverse weather conditions prevented spraying. On this date, results from the first 3 days of aquatic monitoring were thoroughly evaluated. The Project Leader, after reviewing the results, instructed operations personnel to widen the nonspray area to 400 feet on each side of streams. They were told to get application within the specified zone. Idaho Fish and Game Department biologists requested the nonspray area be widened to 600 feet but agreed to 400 feet until additional monitoring could be done.

Spray operations continued on July 6, with helicopters spraying between 400 and 800 feet and planes spraying beyond 800 feet. From July 6 through July 13, 23 stations were monitored.

No adverse aquatic insect losses were detected at the four stations that represented helicopter application. More than 400 percent increase in drifting aquatic insects occurred at six of the 11 stations representing TBM applications but at neither of the two stations in the B-25 application areas.

At five stations, both helicopters and TBM planes were spraying in the same drainage on the days streams were monitored. Each of the five stations had more than a 400 percent increase in drifting aquatic insects, but it was not possible to assign each loss to a particular type plane.

Idaho Fish and Game Department biologists requested further withdrawals from streams. Based on the above information, this seemed justified for planes but not for helicopter spraying. Thus, adjustment of fixedwing specifications was recommended to the Project Leader who agreed and ordered pilots of fixed-wing spray planes to move back beyond a minimum of 1200 feet from streams.

Adverse results on July 14, using the 400and 1200-foot control specifications, required further modification in specifications. On July 15, spray plane pilots were ordered to move their operations back to 1400 feet. Helicopter pilots were ordered back to 600 feet to more completely spray the area between 600 and 1400 feet. During the remainder of the project, the 600- and 1400-foot protection specifications were used.

Changes in stream protection measures which were instituted during the project are illustrated in figure 17.

A summary of the results of spraying in different types of planes for these various zones is indicated in this figure.

The number of samples as related to types of aircraft at various stream protection distances are, in some instances, too few for conclusive results. From the data shown in tables 4, 5, and 6, however, it appears that helicopter spraying with 0.5 pound DDT per acre using 400- and 600-foot protection nonspray zones, did not cause any significant increase in drifting aquatic insects. It also appears that, in this project, TBM aircraft operating 1400 feet or more from the stream while applying 1 pound of DDT per acre maintained a minimum impact on aquatic insects. In all cases, however, strict compliance with all protection control specifications was necessary to keep aquatic insect losses at a minimum.

Porphyry Creek Special Study

Monitor personnel recognized that streams could conceivably become contaminated from one or a combination of several sources. Drifting spray, leaky or faulty spray nozzles, and direct spraying into a protected stream or tributaries because of pilot error were possibilities. Direct spraying into unprotected tributary feeder streams could also carry contaminants into protected streams. A limited investigation was initiated to check possibilities of this later source.

Porphyry Creek typifies many streams within the spray area. The main stream stems from two major forks, each of which is fed by

Figure 17. Illustration of chronology of changes in stream protection measures and types of spray craft and stream protection effectiveness.

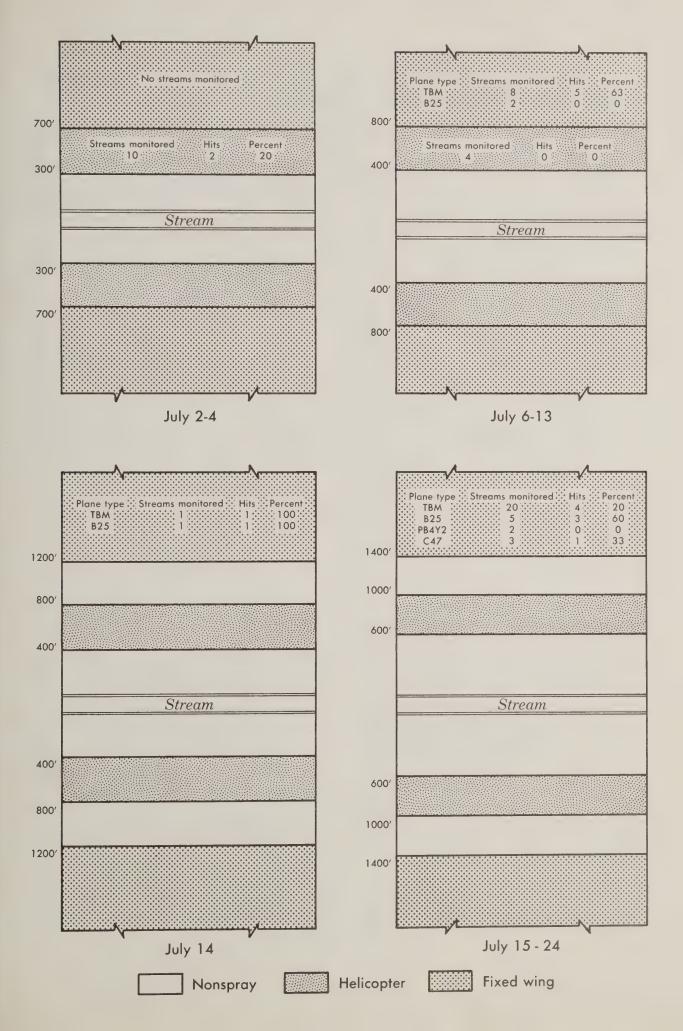
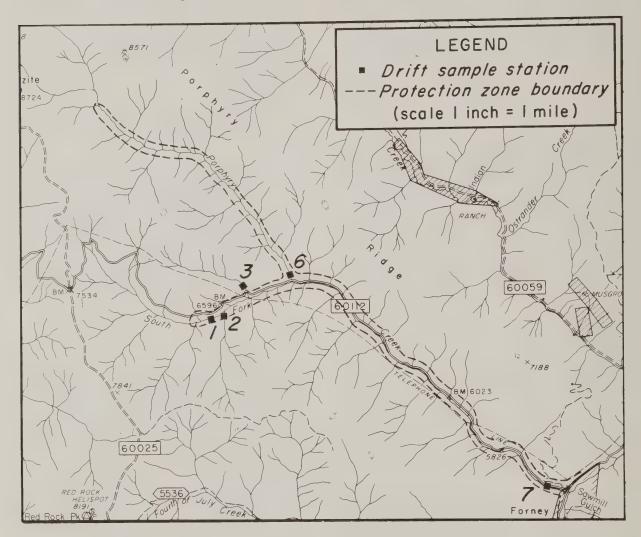


Figure 18. Porphyry Creek Special Study Area. The locations of aquatic insect drift sampling stations and stream protection zones are shown.



many small tributaries (fig. 18). Two drift sample stations, numbers 2 and 3, were established at the lower ends of two unprotected tributaries. Both streams had less than 1 cfs flow. Water was flowing only in the lower 500 feet of the stream on which station number 2 was located. Three stations, numbers 1, 6, and 7, were established on protected sections of Porphyry Creek. Results of aquatic insect drift sampling for all Porphyry Creek stations are contained in table 4.

On July 15, helicopters sprayed in this drainage, observing a 600-foot nonspray zone on each side of the stream. The results showed that large numbers of aquatic insects were killed at station 3, while other stations showed no increased insect drift. On July 16, the

PB4Y2 sprayed the north side of Porphyry Creek. Only station 3 showed a significant increase in insect drift.

Analysis of data indicate the probability that aquatic insects in most small unprotected streams are affected by spray application. It is interesting to note, however, that although station 3 showed a high percent increase in drifting insects on both dates, station 7, located approximately $4\frac{1}{2}$ miles downstream, did not show corresponding increases. Apparently spray materials in the stream at station 3 did not reach station 7 in concentrations great enough to cause an increase in numbers of drifting aquatic insects. Increased numbers of drifting insects from station 3 did not reach station 7.

EVALUATING FISH AND AQUATIC HABITAT

Bottom Insect Sampling

A limited number of streams were selected to study prespray and postspray aquatic insect populations. Because of the large number of streams which showed insect mortalities during spraying operations, as determined by drift sampling, additional streams were added for bottom sampling (fig. 19). Table 8 summarizes bottom sample data collected.

Bottom sampling areas were selected in the lower reaches of each stream. Riffles of sufficient size to accommodate the planned sampling and having uniformity of gravel size, water depth, and flow velocity were selected. Riffle areas selected for sampling were divided into two or three sections according to the number of planned sampling periods. Each section was sufficiently large to allow the collection of five 2-square-foot samples, or a total of 10 square feet per sampling station. Samples were taken moving progressively upstream to avoid disturbing areas to be sampled.

The sampling apparatus consisted of a cylinder shape frame with both ends open. One-half the perimeter was covered with screening of 144-mesh and the other half with hardware cloth of 36-mesh. End openings were 2 square feet in size. When used, the coarser mesh side was placed upstream thus allowing water to flow rapidly enough through the upstream side to hold insects against the smaller mesh on the lower side. To sample, an open end was placed firmly into the bottom gravel of the stream. A wooden paddle was used to stir the gravel inside the case and dislodge the insects.

Insects collected from bottom samples were placed in glass vials of alcohol. Collection information was recorded on the same field record form as used for drift sampling (fig. 14). Vials were labeled on the outside with felt marker pens. Paper labels with penciled numbers were placed inside.

Quantitative taxonomical identification was completed on all bottom samples by Forest Service entomologists and fisheries biologists.

Insects from both the prespray and first postspray samples were keyed to families. The last postspray samples, however, were keyed only to orders.

Bottom sampling data in table 8 shows a general increase of aquatic insect population numbers within 3 months after spraying. Without more prespray data, however, it is not possible to determine the degree of population recovery. A more complete evaluation of population dynamics would require longer-term studies. To establish a base, sample data should have been collected about the same dates at least 1 year before spraying. General findings shown in table 8 agree with findings of Cope (1961) and Schoenthal (1963).

In three of the five project streams and both control streams, prespray insect population numbers were less than October post-spray numbers. A partial explanation may be that egg hatches were incomplete at the June sampling; but by October, hatches were completed and aquatic insects were in more advanced stages of development. Also, low water levels in October tend to concentrate insect populations. Recruitment takes place from unsprayed upper-stream waters and can account for possible population variations.

Prespray populations sampled within the spray area averaged approximately 34 percent *Ephemeroptera*, 5 percent *Plecoptera*, 55 percent *Trichoptera*, 6 percent *Diptera*, and less than one-half of 1 percent *Coleoptera*.

October postspray populations consisted of approximately 24 percent Ephemeroptera, 30 percent Plecoptera, 4 percent Trichoptera, 40 percent Diptera, and 2 percent Coleoptera. These findings indicate a considerable reduction in the percent composition of Ephemeroptera and Trichoptera populations. Conversly, both Plecoptera and Diptera increased. The decline in percentage composition of Trichoptera appears typical as evidenced in other studies. The decline in Ephemeroptera as compared to an increase in Plecoptera is somewhat unusual, however, although it has happened in other instances as shown by

Table 8. Classification of aquatic insects collected as bottom samples (Numbers of insects, by orders, collected per 10 square feet per stream sampled)

			June, Prespray	e, 1964 ay numbers	ers				July, 1964 Postspray numbers	July, 1964 spray num	4 nbers			Pc	Octob	October, 1964 Postspray numbers	ers	
Drainage and stream	Ephemer- optera	Ple- coptera	Tri- choptera	Diptera	Cole- optera	Totals	Ephemer- optera	Ple- coptera	Tri- choptera	Diptera	Cole- optera	Totals	Ephemer- optera	Ple- coptera	Tri- choptera	Diptera	Cole- optera	Totals
Main Salmon River			ţ	1	4												(į
Boulder Creek								13	25	_	9	46	4	34		က	2	47
Colson Creek							53		2	က	က	99	40	131	53	12	=	247
Garden Creek							1	9	208		4	229	65	84	105	4	12	270
Indian Creek							44		00	2	-	58	245	64	29	881	18	4442
Owl Creek								4	4	-	က	-	4	41	2	761	က	126^{2}
Pine Creek	23	-	5	m	-	33	44		=	က		62	27	88	53	1041	က	275^{2}
Spring Creek	1.5	Ser.					52	-	12	4	4	73	73	14	25	œ	00	128
North Fork of Salmon River	1																	
Dahloneaa Creek	1						4	10	2	က	1	19	16	58	1	10	-	85
lower No. Fork	53	-	2	9		61	ന	3 21	6	5	_	÷ 39	114	42	11	1281	-	2962
Pierce Creek							22		10	_	1.	44	160	81	က	16	က	263
,	179	16	167	39	် က	404	37	7 3	22		_	64	38	43	1	13	1	94
Panther Creek																ψ.		
Copper Creek							Eb.		13	10		25	57	∞	0	1401	-	215^{2}
Deep Creek							31.95	11	84	2	4	196	89	42	14	2001	-	370^{2}
Lower Panther			-	١		-				1,4		1,4			_	1881		1892
Creek	7	1 6	- 4	- S - S - S		2 (4	1	-		2 1	50	13	240	317	۱)) (0	564
Moccasin Creek							יין הו ריים		0		177	0 0	46	167	_	2881	· /	5152
Moyer Creek							7 2		1 1	-		7.7	0 0	42))	۰ ،	77
reek		o ·					4 c		, , ,			217	2 9	747	- 22] 0	2 7	γ1α
	133	38	466	<u> </u>	1	7.00	22	200	771	-		417	60	404	\ 0	-	-	0
Panther (above Blackbird)				, d			7 8.5		2	7	က	20	16	58	-	3441	1	4182

Table 8. (continued)

	Totals		5	-2	. r	22	1 40	6 ²		2		0.	_	_	က	0			2 ₂ 2
	Cole-		255	- Para	(r)	2		9462		3912			471		23	110			285 588 ²
54 bers	optera		က	-			10	15	16			٥	- 20	2	2	5			31
October, 1964 stspray numbe	Diptera		35	12561	_	2	7	5121	٥	3881		22	25	5	9	4			31
October, 1964 Postspray numbers	Tri- choptera		22	1	-	1]	19	12	-		-	12	-	1	12			389
~	Ple- coptera		103	က	18	က	37	302	134	_		119	201	21	14	25		,	13
	Ephemer- optera		92	_	_	17	9	86	197	-		48	215	198	_	64		!	83
	Totals		55	33	30	26	30	58	76	94		48	70	61	11	33		0	309
4 nbers	Cole- optera		1	1	2	72	က	1	1	-		7	-	_	1	1			
July, 1964 Ispray num	Diptera		- 1	-	1	-	-	2	_	1		17	œ	32	-	7		(179
July, 1964 Postspray numbers	Tri- choptera		2	25	24	9	5	7	73	06		2	21	œ	က	17			361
	Ple- coptera		œ	2	က	2	16	6	٥	1		က	5	-	7	က		:	7 2
	Ephemer- optera		45	5	_	12	9	40	14	က		24	35	19		Ξ		:	182
																		1	. 0
	Totals Cole-																	ć	150
1964 numbers	optera				P.														ري
	Diptera																	Č	7
June, Prespray	Tri- choptera		5												5			7	71
	Ple- coptera																	1	=
	Ephemer- optera																	101	69
	Drainage and stream	Panther Creek (cont.)	Panther (above Opal)	Panther (above Napias)	Panther (above 4th of July)	Panther (below 4th of July)	Trail Creek	Woodtick Creek	4th of July (above dump)	4th of July (below dump)	Camas Creek	Beagle Creek	Meadow Creek	Shovel Creek	Silver Creek	Yellow Jacket Creek	Lemhi River		Haynes Creek

¹Because of the large number of Diptera in these samples, only an estimated 25 percent were picked and counted. Therefore, each noted figure has been expanded from the estimate.
²Totals include expanded Diptera counts.



Figure 19. Aquatic insect bottom sample locations were as follows: (1) Haynes Creek, (2) Geertson Creek, (3) Dahlonega Creek, (4) Upper North Fork Salmon River. (5) Pierce Creek, (6) Lower North Fork Salmon River, (7) Indian Creek, (8) Squaw Creek, (9) Spring Creek, (10) Boulder Creek, (11) Pine Creek, (12) Owl Creek, (13) Colson Creek, (14) Garden Creek, (15) Lower Panther Creek, (16) Trail Creek, (17) Moccasin Creek, (18) Panther Creek (above Naipas Creek), (19) Deep Creek, (20) Panther Creek (above Blackbird Creek), (21) Copper Creek, (22) Woodtick Creek, (23) Musgrove Creek, (24) Moyer Creek, (25) 4th of July Creek (above PB4Y2 dump), (26) 4th of July Creek (below PB4Y2 dump), (27) Panther Creek (below 4th of July Creek), (28) Panther Creek (above 4th of July Creek), (29) Opal Creek, (30) Panther Creek (above Opal Creek), (31) Silver Creek, (32) Yellowjacket Creek, (33) Shovel Creek, (34) Beagle Creek, (35) Meadow Creek.

Graham and Scott.¹ Differences in *Plecoptera* insect size and genera apparently make a considerable difference in susceptibility to insecticides (Jensen and Gaufin 1964b).

In one instance, at the station located on the upper North Fork of the Salmon River, there was a considerable decrease in population numbers. The highest concentrations of DDT in water samples were collected at this station. The flushing time or velocity of stream flow also affected DDT concentrations at this particular station. The confluence of Pierce Creek with the North Fork of the Salmon River is only a few hundred feet upstream from where the North Fork bottom sampling station was located. The lower end of Pierce Creek has numerous beaver dams and ponds. When the Pierce Creek drainage was sprayed, drift insect counts were very high and a correspondingly high count was obtained at the North Fork sampling station. Apparently, DDT spray which reached the water in Pierce Creek was flushed downstream slowly but continuously. This was substantiated when 2 days later, drift samples taken before any spraying was done on that day still showed larger than normal numbers of insects.

Some researchers feel that recruitment of aquatic insects from unsprayed portions of stream headwaters is important from the standpoint of repopulation. Schoenthal (1963) found that repopulation took place by downstream drift recruitment from the unsprayed section of a stream above, as well as from new egg deposition. A comparison of data in table 8 with proportions of drainages sprayed indicated, however, no direct correlation between postspray populations and percentage of stream sprayed. Actually, in some streams where the entire drainage was sprayed, and where heavy insect losses occurred, both high and low repopulation numbers showed up in the October bottom sampling. In all cases, however, streams with only a short section of the lower portion of the drainage sprayed showed substantial increases in population from July to October.

Water Sampling

Water samples were collected from several locations (fig. 21) to determine the amount of DDT in the streams.

Sampling procedures utilized two different techniques. Most were dip samples taken by immersing a collection container in the water and allowing it to fill as quickly as possible. All dip samples were taken near the center of the main current with the openings of the container held a little below the surface. The second method involved the test use of burettes to obtain a continuous sample over a given period of time. A discussion of burette use, and a comparison of the two methods, is included later in this report.

Water samples were collected in either 1-pint or 1-gallon tin cans. Identification numbers were written on the cans, collection information recorded on field record sheets (fig. 22), and the samples sent to the Agricultural Research Service. Laboratory. There they

¹Graham, R. J. and Scott, D. O., Effects of an aerial application of DDT on fish and aquatic insects in Montana, 1959. (Joint project report by Montana Fish and Game Department and U.S. Forest Service, on file with Montana Fish and Game Department, Helena, Montana.)













Figure 20. A bottom sampler is cylinder-shaped with both ends open. One-half the side is covered with 144 mesh screen and the other half with 36 mesh screen. It is placed in the stream with the coarse mesh upstream and the stream bottom stirred thoroughly. The debris is washed into a dishpan where the insects can be picked out and placed in vials of alcohol. Large rocks from the sample are examined for insects. Five samples of 2 square feet each, a total of 10 square feet, are taken at each station.



were analyzed for DDT residues according to the following procedure, quoted from the analysis report.¹

Upon receipt at the Laboratory, the cans were stored under regular refrigeration temperatures.

At analysis time, the cans were shaken to mix their contents and the volume of water in the can determined by actual measurement. An aliquot of the total volume was taken (usually 1,000 ml.) and the can rinsed with a solvent and a like aliquot of the rinsings taken and combined with the original aliquot taken for analysis.

The insecticide content of the water was removed by repeated liquid-liquid extractions with distilled n-hexane. The solvent extracts were combined for each sample and evaporated to dryness and made to a definite volume.



The insecticide content of the solvent extracts of the water samples was determined by gas chromatography. A lower limit of accuracy of the analytical method was established at 0.2 part per billion.

Known amounts of insecticides were added to water samples, after original analysis indicated that the residue level in the sample was low, and the percentage recovery of the amounts added was determined. The average recovery was 93.5%, with a range from 80 to 100 percent. Residue level added ranged from 0.5 to 1.0 part per billion.

Originally, plans provided for collection and analysis of approximately 85 water samples. Eventual expansion of monitoring plans resulted in the collection of 446 water samples. Therefore, 149 of these samples were selected on a priority basis for analyses (table 9). Water samples selected for analysis were primarily those taken from streams showing significant increase in drifting aquatic insects. Twenty-six samples were found to contain

¹Agricultural Research Service mimeographed report PCY-64-22, 1964. (On file with Entomology Research Div., ARS, Yakima, Wash.)



Figure 22. Water sample collection field record form.

Date	Sample Collector
Station Number	Sample Analyzer

Sample Number	Time	D.D.T. Concentration	Comments
			\

measurable amounts of DDT. None of the 149 samples, however, showed any measurable amounts of DDD (TDE) or DDE. Neither the prespray samples from Opal Creek nor the control samples from Haynes Creek contained measurable amounts of DDT. Two-tenths part per billion was the lowest amount detectable (fig. 23) by the analytical method used.

Usually, when a series of water samples was found to contain DDT, a corresponding increase was noted in the number of drifting insects. This was not always true, however.

Figure 21. Water sample stations were located at: (1) Haynes Creek (control), (2) 4th of July Creek (Salmon River), (3) Upper North Fork Salmon River, (4) Hughes Creek, (5) Hull Creek (east fork), (6) Hull Creek (spring on west fork), (7) Hull Creek (reservoir), (8) Lower North Fork Salmon River, (9) Pine Creek, (10) Boulder Creek, (11) Garden Creek, (12) River Station, (13) Lower Panther Creek, (14) Panther Creek (above Napias Creek), (15) Panther Creek (above Blackbird Creek), (16) Panther Creek (above Musgrove Creek), (17) 4th of July Creek (above road bridge), (18) Opal Creek.

As shown in table 9, water samples R-84 and R-85 (Salmon River station on July 10) contained measurable amounts of DDT, but no corresponding increases of drift insects were noted (table 7). Also, not all streams which showed an increase of drifting insects showed measurable amounts of DDT in the corresponding series of water samples. This was most likely because spray could have passed the sampling point as a block of contaminant in a matter of just a few minutes. Samples taken at hourly intervals could have missed these blocks, similar to that indicated by Cope (1961) in his study on the Yellowstone River.

Water samples collected from Hughes Creek on July 9, as shown in table 9, appear to substantiate the fact that introduced spray from a normal treatment may pass downstream in blocks. In this instance the data show an apparent 1½-hour spacing between blocks.

By explanation, it should be pointed out that water samples collected at the Salmon River station were composites of dip samples. Two pint samples collected every 15 minutes were combined each hour to make a 1-gallon composite hourly sample.

Table 9. DDT residues in stream water samples

Table 9. (continued)

Remarks									Heavy insect drift started at 6:30 a.m.								Insect drift decreased to normal at 10:00 a.m.				Domestic water supply taken after area was	sprayed.	Sample taken day after severe insect mortality.	Sample taken day after severe insect mortality.						
Type of sample	Burette	"	"	"	Dip	Burette	Dip	Burette	Dip	"	Burette	Dip	2	Burette	Dip	"	Burette	Dip	"	Burette	Dip		"	"	"	"	"	"	"	"
DDT (ppb) 2			.32	1		.21	1	09.]	.22	1	1	1	.24	1]	1					1		1	-	1	[1	
	a.m.	p.m.	"	"	a.m.	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"		n	p.m.	a.m.	"	p.m.	n n	a.m.	"
Sample	11:45-12:00	12:00-12:15	12:15-12:30	12:30-12:45	5:20	5:25-6:22	6:20	6:24-7:15	6:50	7:20	7:16-8:24	7:50	8:20	8:25-9:25	8:55	9:25	9:26-10:24	10:25	11:27	10:25-11:26	7:10		10:10	4:45	4:15	8:15	12:15	4:15	4:15	8:15
Sample	A-59	A-60	A-20	A-21	51	1-X	52	2-X	53	54	3-X	55	56	4-X	57	58	2-X	59	90	X-9	None		None	None	73	74	7.5	76	77	78
Date (1964)	6-7				7-11																7-4		7-5	7-5	7-8					
Stream name	Hughes Creek	(continued)			Hughes Creek																Garden Creek		Boulder Creek		Haynes Creek	(Control Station)				

Table 9. (continued)

Remarks		East Fork of Hull Creek.	Spring on West Fork of Hull Creek. Reservoir.	East Fork of Hull Creek.	Spring on West Fork of Hull Creek.	Reservoir.				Heavy insect drift commenced at 7:00 a.m.		Drift count peaked at 9:00 a.m.					Normal nocturnal activity increase noted between	10:00 p.m. and 2:00 a.m.	No increase of drift insects occurred on this date.								
Type of sample	Dip "	: :	*	*	*	*	"	"	"	"	"	"		"	*	*	"		*	"	"	"		"	"	,,	"
DDT (ppb) ²	1	1		1	1	İ	i	1	1	1		1	1	I	-	I	1		1	ļ	.22	.32	1	Ī	Ī	1	ı
	p.m.	a.m.	*	p.m.	"	*	a.m.	"	"	=	"	"	*	"	p.m.	×	*		a.m.	"	2	"	"	ш	"	p.m.	=
Sample time	12:15	3:30	3:35	3:00	3:15	3:25	4:15	5:15	6:10	7:05	8:05	9:05	10:00	11:00	12:10	7:00-8:00	10:00-11:00	1	1:00-2:00	4:00-5:00	5:00-6:00	6:00-7:00	7:00-8:00	8:00-9:00	10:00-11:00	1:00-2:00	4:00-5:00
Sample numbers	79	61	63	64	65	99	133	134	135	136	137	138	139	140	141	R-74	R-77	1	R-80	R-83	R-84	R-85	R-86	R-87	R-89	R-92	R-95
Date (1964)	7-8	7-8					7-8									7-9			7-10								
Stream name	Haynes Creek (Control Station) (continued)	Hull Creek &					Pine Creek									River Station											

Table 9. (continued)

4:05 a.m. — Dip 9:13 " — Dip 9:13 " — " " " " " " "						1	9	
ver) 7-12 121 4:05 a.m. — Dip reek) 7-18 126 2:05 p.m. — " reek 7-17 123 5:20 " — " 124 5:40 " — " 127 6:00 " — " 7-18 125 2:40 " — " 110 4:30 " — " 111 5:30 " — " 111 5:30 " — " 112 5:30 " — " 113 7:30 " — " 114 7:30 " — " 115 8:30 " — " 116 8:30 " — " 117 9:30 " — " 118 9:30 " — " 119 10:15 " — " 85 11:30 p.m. — " 87 12:30 p.m. — " 88 2:30 " — " 88 2:30 " — " 88 2:30 " — " 88 2:30 " — " 88 2:30 " — " 88 2:30 " — " 88 2:30 " — " 88 2:30 " — " 88 2:30 " — " 88 2:30 " — " 88 2:30 " — " 88 2:30 " — " 88 2:30 " — " 88 2:30 " — " 89 3:30 " — " 80 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	Stream name	(1964)	numbers	time		(ppb) 2	sample	Remarks
reek) 7-18 126 2:05 p.m. — " 124 5:40 " — " 127 6:00 " — " 127 6:00 " — " 7-18 125 2:40 " — " 110 4:30 a.m. — " 111 5:30 " — " 111 7:30 " — " 112 8:30 " — " 114 7:30 " — " 115 8:30 " — " 116 8:30 " — " 117 9:30 " — " 118 9:30 " — " 118 9:30 " — " 119 10:15 " — " 110 11:30 " — " 111 9:30 " — " 112 8:30 " — " 113 8:30 " — " 114 7:30 " — " 115 8:30 " — " 116 8:30 " — " 117 9:30 " — " 118 9:30 " — " 119 10:15 " — " 110 11:30 " — " 111	4th of July (Salmon River)	7-12	121	4:05	a.m. "		Dip "	2.6 miles upstream from Salmon River highway.
7-17 123 5:20 " — " Above mouth PB4Y2 dump. 124 5:40 " — " Above mouth PB4Y2 dump. 127 6:00 " — " Above mouth Oump. 128 125 2:40 " — " One-half mile July Creek in respectively of the part of the par	4th of July (Panther Creek)	7-18	126	2:05	ë. G	Ţ	*	Above Panther Creek road bridge on 4th of July Creek in relation to jettisoned PB4Y2 load.
7-18 125 5:40 " — " 7-18 125 2:40 " — " 7-11 109 4:30 a.m. — " 111 5:30 " — " 113 7:30 " — " 114 7:30 " — " 115 8:30 " — " 116 8:30 " — " 117 9:30 " — " 118 9:30 " — " 119 10:15 " — " 120 10:30 " — " 85 11:30 " — " 87 12:30 p.m. — " 88 2:30 " — " 88 2:30 " — " 88 8 2:30 " — " 89 3:30 " — " 88 8 2:30 " — " 89 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	Panther Creek	7-17	123	5:20		1	*	Above mouth of Musgrove Creek in regard to PB4Y2 dump.
7-18 125 2:40 "			124	5:40	2	1	"	
7-18 125 2:40 "			127	9:00	×	I	*	
7-11 109 4:30 a.m. — "" 110 4:30 "" — "" 111 5:30 "" — "" 112 5:30 "" — "" 113 7:30 "" — "" 114 7:30 "" — "" 115 8:30 "" — "" 116 8:30 "" — "" 117 9:30 "" — "" 120 10:30 "" — "" 85 11:30 "" — "" 86 12:30 "" — "" 87 2:30 "" — "" 88 2:30 "" — "" 89 3:30 "" — ""		7-18	125	2:40	*	1	*	One-half mile downstream from mouth of 4th of July Creek in regards to PB4Y2 dump.
110 4:30 "	Upper North Fork	7-11	109	4:30	a.m.	1	"	
111 5:30 "" — "" 112 5:30 "" — "" 113 7:30 "" — "" 114 7:30 "" — "" 115 8:30 "" — "" 116 8:30 "" — "" 117 9:30 "" — "" 118 9:30 "" — "" 120 10:30 "" — "" 121 11:30 "" — "" 122 12:30 "" — "" 1230 85 12:30 "" — "" 1230 87 12:30 "" — "" 124 "" 125 12:30 "" — "" 126 12:30 "" — "" 127 128 88 2:30 "" — "" 128 12:30 "" — "" 129 12:30 "" — "" 120 120 120 120 120 120 120 120 120 120	Salmon		110	4:30	"	1	"	
5:30 "			111	5:30	"	1	2	
7:30 "			112	5:30	"	1	"	
7:30 "			113	7:30	"	1	"	
8:30 " .44 " 8:30 " .24 "24 "24 "29			114	7:30	"	ı	"	
8:30 "			115	8:30	"	.44	"	
9:30 "24 ". 9:30 "29 ". 10:15 "22 ". 11:30 " 12:30 p.m 12:30 " 2:30 " 3:30 "			116	8:30	"	1	"	
9:30 " .29 " 10:15 " .22 " 11:30 " " .12:30 p.m. — " 12:30 " "			117	9:30	"	.24	"	Large increase in insect drift started at 9:00 a.m.
10:15 " .22 " " .10:30 " " " " "			118	9:30	"	.29	"	
10:30 " " " " " " " " " " " " " " " " " " "			119	10:15	"	.22	"	
11:30 " " " " " " " " " " " " " " " " " " "			120	10:30	"	1	"	
12:30 p.m			85	11:30		1	,,	
12:30 " — " 2:30 " — " 3:30 " — "			86	12:30	p.m.	İ	,,	
2:30 " " " " " " " " " " " " " " " " " " "			87	12:30	"	Ī	"	
3:30 " " "			88	2:30	"	I	"	
11.30 "			89	3:30	"	1	"	
5:4			06	4:30	"	Ī	,,	Heavy insect drift continuing.

Table 9. (continued)

Remarks	A heavy insect drift was noted from 4:00 a.m. to	4:00 p.m. when sampling stopped. Observers re-	ported both helicopter and TBM spraying above	this station. Acute spray drift was noted during	spray operations.																					This series of samples taken in conjunction with the	PB4Y2 dump on 4th of July Creek. This station was	located at the lower end of Panther Creek at live-	box test site.
Type of sample	Dip	"	"	n n	"	n n	"	"	*	"	"	"	ĸ	"	"	"	"	"	"	п	"	"	"	"	"	"	и	"	n n
DDT (ppb) ²	1	1.00	.37	.80	.57	.76	1.21	.57	.42	1	1	1	1	Ī	Ī	1	Ī	Ī	1	I	I		1	I	1	1	1	1	1
	a.m.	"	ш	"	n	"	"	"	p.m.	g.B.	"	p.m.	"	a.m.	"	"	"	"	"	ш	p.m.	"	"	"	*		"	"	"
Sample	4:00	5:00	9:00	7:00	8:00	00:6	10:00	11:00	12:00	5:00	8:00	12:00	4:00	5:00	6:10	7:05	8:05	9:10	10:00	11:00	12:06	1:06	2:10	3:00	4:05	12:30	1:00	1:30	2:00
Sample	7-X	X-8	X-6	10-X	11-X	12-X	13-X	14-X	15-X	91	92	93	94	79	89	69	70	71	72	67	86	66	100	101	102	Panther #1	Panther #2	Panther #3	Panther #4
Date (1964)	7-13									7-123				7-13												7-17			
Stream name	Upper North Fork	Salmon								Opal Creek				Opal Creek												Panther Creek			

'able 9. (continued)

Stream name	Date (1964)	Sample numbers	Sample		DDT (ppb) ²	Type of sample	Remarks
Panther Creek	7-17	Panther #5	2:30	p.m.		Dip	
(continued)		Panther #6	3:00	"	I	"	
		Panther #7	3:30	"	1	"	
		Panther #8	4:00	"	1	n n	
		Panther #9	4:30	"	-	"	
		Panther #10	5:00	"	1	"	
		Panther #11	5:30	"	1	n n	
		Panther #12	9:00	"	1	n n	
		Panther #13	6:30	"		"	

¹ Agricultural Research Service mimeographed report PCY-64-22, 1964. (On file with Entomology Research Div., ARS, Yakima, Wash.)
²All values less than 0.2 ppb shown as "—".

Live-box Testing of Fish Description

In order to detect acute toxicity and fish mortality during spraying operations, fish in live-boxes were placed at selected locations both inside and outside the spray area (fig. 24). Two species of fish, chinook salmon and rainbow trout (Salmo gairdneri), inhabitants of streams in the Salmon area, were tested to assess the effects or differences in susceptibility to DDT. Both the Forest Service and the Idaho Fish and Game Department participated in this phase of monitoring.

Forest Service. Live-boxes were located on both Haynes and Geertson Creeks as control stations. Test stations were located on the upper and lower ends of the North Fork of the Salmon River, the lower ends of Panther Creek, and Opal Creek, and on the main Salmon River near the mouth of Ebenezer Creek.

Live-box dimensions were approximately 4' x 1½' x 1½'. Boxes used to hold chinook salmon were covered with aluminum 144 mesh window screening. Boxes containing rainbow trout were covered with ¼-inch mesh hardware cloth. Each box was boarded solid on half the bottom area, one end (upstream end), and both sides. In addition, a louvered partition was built inside the boxes to lesson water current velocity. Louver spacing allowed fish to move freely from one section of the box to the other. The boxes were held in place with wire and stone anchors.

Originally it was planned to place 200 fish in each box 4 days prior to spraying. On the day before spraying, 100 fish were to be removed leaving 100 of the most vigorous fish. This would eliminate weak and dead fish resulting from transportation and handling. The number of chinook salmon available for testing was limited, however, and fewer fish were used. It was also necessary in some cases, to use a shorter tempering period prior to spraying, because of difficulties in correlating personnel work schedules with spraying schedules.

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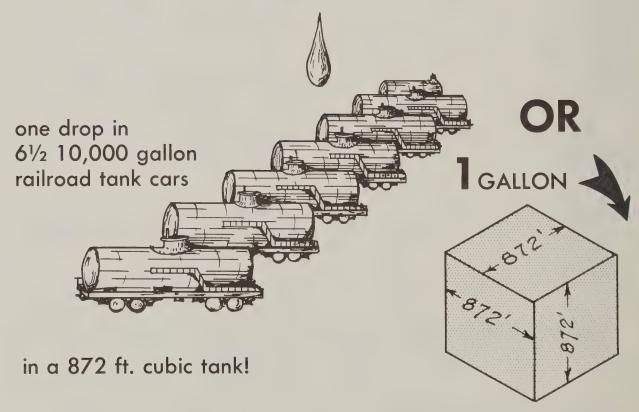


Figure 24. Forest Service live-box stations were located at: (1) Haynes Creek (control station), (2) Geertson Creek (control station), (3) Upper North Fork, (4) Lower North Fork, (5) Main Salmon River, (6) Lower Panther Creek, (7) Opal Creek. Idaho Fish and Game Department live-boxes were located at: (2) Geertson Creek (control station), (8) Panther Creek, (9) Indian Creek (10) North Fork Salmon. Wild fish sampling locations were on: (11) Lemhi River (control location), (12) Dahlonega Creek, (13) Lower North Fork Salmon River, (14) Indian Creek, (15) Squaw Creek, (16) Spring Creek, (17)

Main Salmon River, (18) Colson Creek, (19)
Panther Creek, (20) Musgrove Creek, (21)
4th of July Creek (above PB4Y2 dump),
(22) 4th of July Creek (below PB4Y2 dump),
(23) Panther Creek (below 4th of July
Creek), (24) Panther Creek (above 4th of
July Creek). Aquatic vegetation sampling locations were on: (4) North Fork Salmon River, (9) Indian Creek, (11) Lemhi River, (12)
Dahlonega Creek, (16) Spring Creek, (18)
Colson Creek, (20) Musgrove Creek, (25)
Boulder Creek, (26) Moose Creek, (27) Wagonhammer Creek.



Test fish consisted of rainbow fingerlings 2 and 4 inches long, provided by the Idaho Fish and Game Department Mackay Fish Hatchery. Chinook salmon fingerlings 2 to 3 inches long were collected from downstream migration traps within the Lemhi, Salmon, and Pahsimeroi Rivers. A few chinook salmon were collected by use of an electric shocker from the Lemhi River.

Test fish were transported to live-box stations in fish planting trucks. Water temperatures were controlled by icing when necessary. Every effort was made to equalize water temperatures when the fish were placed in live-boxes.

During the period of spray applications, live-boxes were checked daily when possible. Observations of mortality, water level conditions, and in some cases water temperatures were recorded on field record forms (fig. 26). Individual records were kept on each species of fish for each station.

Idaho Fish and Game Department. Additional live-boxes were installed by the Department (fig. 24). These live-box studies were in conjunction with the study to evaluate long-term effects on fish subjected to sublethal amounts of DDT.

Test fish used in these studies were also obtained from the Mackay (Idaho) Fish Hatchery. Rainbow fingerlings approximately $2\frac{1}{2}$ to $3\frac{1}{2}$ inches long were placed in Panther Creek, in Indian Creek, and controls in Geertson Creek. Adult rainbow trout averaging $11\frac{1}{2}$ inches long were placed in Panther Creek, Indian Creek, in two sections of a rearing channel in the North Fork of the Salmon, and as controls in Geertson Creek.

Fish used in the Lower North Fork studies were not placed in live-boxes. In this case, the fish were held in short sections of a side channel of the North Fork. This was an artificial channel with regulated water flow.

Live-boxes used by the Department measured 4' wide x 8' long x 3' deep. The top of each box was covered with a black colored plastic sheet to protect the fish from direct sunlight. Each box was anchored in slack water or eddies to reduce swim fatigue.

Figure 25. Chinook salmon and rainbow trout were held in fish live-boxes on a number of spray project streams and control streams from before spraying through a testing period after spraying.



Results

Forest Service. Fish in the control boxes located on Haynes and Geertson Creeks showed a low mortality rate for both species, (tables 10 and 11). The control fish received the full 4-day tempering. Control boxes were located so they did not become subjected to fluctuating water levels. Water temperatures ranged in the mid-forties on both streams.

The Opal Creek live-box test was limited to rainbow trout, as chinook salmon were not available. A 1-day tempering period resulted in only one dead fish. Due to work schedules,

Figure 26. Live-box field record form.

Fish	in	Live	Box.				
Stati	on	Nur	nber	Marie 1971			

Date	Time	Number Alive in Box	Number Dead Removed	Comments (Include Recorder's Name)
,				,

the usual culling to 100 test fish was not done. Five fish, of 280 fish placed in the live-box, died during the testing period. It is doubtful that the mortality sustained in this box can be attributable directly to the spray operation.

The main Salmon River test station showed only limited rainbow trout mortality but chinook salmon losses at this station were extreme. Most of the rainbow loss, 14 of 20, occurred on 1 day, July 19. On that date the river level dropped, lowering water in the rainbow trout live-box to only 1 inch deep. Fish were extremely crowded and this factor is probably responsible for the loss. The number of chinook salmon used in the Salmon River test was small. These fish were also subjected to a sudden drop in water level, which likely contributed to their high mortality rate.

Results from the lower and upper North Fork of the Salmon River live-box tests indicate survival was excellent for both species of fish. The lower station was subjected to fluctuating levels although water was never less than 6 inches deep inside the live-boxes.

Survival results for the lower Panther Creek station were 89 percent for rainbow trout and 60 percent for chinook salmon. Water level in the Panther Creek boxes dropped several inches overnight on several occasions, necessitating moving the boxes. On some days the water in the boxes was down to 2 or 3 inches in depth. Daily records shown in tables 10 and 11, show no definite patterns indicating a buildup of mortality from any specific cause. In both boxes some mortality occurred after the tempering period but before spraying was done upstream from the test station. From these data it is difficult to identify the exact cause of mortality, but it does not appear to be caused by spray exposure.

Idaho Fish and Game Department. The results of the rainbow fingerling studies are shown in table 12. Losses in Geertson Creek, the control stream, were small until the latter part of the testing period. In both the Geertson and Indian Creek boxes, the heavier losses sustained during the latter part of the testing period were probably caused by starvation. Losses in the Panther Creek box, however,

Table 10. Rainbow trout mortality in live-box tests by the Forest Service

Date	C	ontrol	streams						Test st	reams				
	Нау	nes .	Gee	rtson		wer Fork	Lower	Panther	· O	pal	Up Norti	per Fork		ain n River
July 1964	Live fish in box ¹	Dead fish removed	Live fish in box ¹	Dead fish removed	Live fish in box ¹	removed Dead fish	Live fish in box ¹	Dead fish removed	Live fish in box ¹	Dead fish removed	Live fish in box1	Dead fish removed	Live fish in box1	Dead fish removed
1	100		100				100							
2	100		100				100						2	
3	100		100				99	1						
4	100		100				98 ⁶	1						
5	100		100		100		97	1						
6	100		100		100 ²		95	2						
7	100		100		100		945	1					200	
8	100		100				94						199	1
9	100		100		100		93	1					199	
10	100		100										199	
11	100		100		100 ⁵		92	1			100 ²		199	
12	100		100		100		92		280		100		199	
13	100		100		100		91	1	279 ²	1	100		197	2
14	100		100		100		91 ⁵		278	1	100		195	2
15	100		100		100 ⁵		89	2			100		100 ³	95³
16					100				278		100		99	1
17	100		99	1	100		89				100			
18														
19	100		98	1	100		89 ⁵				100		85	144
20									278					
21	100		98		99	1	89				100		85	
22	End o	f testing	g perio	d					277	1				
29							End of	test	275	2				
Totals	100	0	98	2	99	1	89	_ 11	275	5	100	0	195 ⁷ 85 ⁸	5 15
Percent		0		2		1	1	1		2	()	2.57 8	k 15 ⁸

¹Blanks in these columns indicate live-boxes not inspected on those dates.

NOTE: Tempering period for the Haynes and Geertson Creek stations began on June 27.
Tempering period for Panther and lower North Fork stations began on June 28.
Tempering period for upper North Fork station began on July 6.
Opal Creek received a 1-day tempering period.
Main Salmon River fish received no tempering period.

²First day spraying occurred upstream from test site.
³95 fish removed because of crowding and dropping water and box was moved to deeper water.
⁴Water level dropped and only one inch of water remained in box.

⁵Live-boxes moved into deeper water due to drop in water level.
⁶First day spraying occurred upstream from test site and live-box moved to deeper water due to drop in water level.

Total at July 15 before 95 fish were removed.

Total left of 100 fish in box on July 16.

Table 11. Chinook salmon mortality in live-box tests by the Forest Service

Date	C	ontrol	treams					Test str	eams			
	Hay	nes	Gee	rtson	Low North		Lower	Panther	Up _l North	er Fork		iin n River
July 1964	Live fish in box ¹	Dead fish removed	Live fish in box ¹	Dead fish removed	Live fish in box ¹	Dead fish removed	Live fish in box ¹	Dead fish removed	Live fish in box ¹	Dead fish removed	Live fish in box ¹	Dead fish removed
1	50		50				50					
2	49	1	50				48	2			2	
3	49		49	1			48					
4	49		49				48 ⁵					
5	49		49		50		48					
6	48	1	49		50 ²		48					
7	48		49		50		45 ⁴	3			10	
8	48		49				45				9	1
9	48		49		49	1	44	1			9	
10	48		49								9	
11	48		48	1	47 ⁴	2	42	2	60^{2}		9	
12	48		48		47		40^{4}	2	60		9	
13	48		48		45	2	39	1	60		6	3 ³
14	48		48		45		35	4	60		6	
15	48		48		45 ⁴		35		60		4	2
16					45				59	1	4	
1 <i>7</i>	48		48		45		32	3	59			
18												
19	48		48		45		30 ⁴	2	59		2 ⁴	2
20												
21	48		48		45		30		59		2	
		of testi	ng perio	d								
Totals	48	2	48	2	45	5	30	20	59	1	2	83
Percent		4		4	1	0	4	.0	:	2	7	5

¹Blanks in these columns indicate live-boxes not inspected on those dates.

Tempering period for the Haynes and Geertson Creek stations began on June 27. Tempering period for the Panther and lower North Fork stations began on June 28. Tempering period for upper North Fork station began on July 6. Main Salmon River fish received no tempering period. NOTE:

²First day spraying occurred upstream from test site.

^{*}Hole in box allowed two of these fish to escape.

4Live-box moved to deeper water due to drop in water level.

5First day spraying occurred upstream from test site and live-box was moved to deeper water due to drop in water level.

were extreme and occurred earlier in the testing period. Physical condition of fish is reflected somewhat in a later discussion regardingextractablelipids of fish analyzed for DDT residues.

The pattern of fish mortality at the Idaho Fish and Game Department Panther Creek station is quite different from that of rainbow fingerlings held in Forest Service live-boxes

at the same location. Forest Service data indicate mortality was spasmodic and could have resulted from water level changes. Objectives of the Forest Service evaluation were for determining acute effects and plans provided for holding fish in live-boxes for only 2 to 3 weeks. Fish in the Forest Service live-box on Panther Creek may have suffered losses similar to Department losses had the fish been held for a longer period.

Table 12. Fingerling rainbow trout mortality in live-box tests by the Idaho Fish and Game Department¹

	Control	stream		Test st	reams	
Inspection Date	Geertso	n Creek	Panthe	r Creek	Indian	Creek
(1964)	Live fish	Dead fish removed	Live fish	Dead fish removed	Live fish	Dead fish removed
6-29					350	
6-30			350		300 ²	
7-2			300 ²			
7-4			3		3	
7-5			300		288	12
7-6	300					
7-8	300		292	8	287	1
7-12			289	3	287	
<i>7</i> -15	300					
7-21			256	33	287	
7-22	298	2				
7-27	298		120	136	287	
8-3	297	1	75	45	287	
8-6			66	9	287	
8-19	297					
8-21			56	10	287	
8-24			55	1	287	
9-8			55		287	
9-14	297		55		287	
9-24	295	2	55		276	11
10-1	293	2			262	14
10-2		_	55			
10-3						
10-7	290	3	55		246	16
10-14	283	7	55		215	31
10-20	261 ⁴	17			169 ⁴	31
10-21		• •	10 ⁴			
	End of test	ing period				

¹From long-range study being conducted cooperatively between the Forest Service and Idaho Fish and Game Department.

2Removed weaker fish and started test period.

3First day spraying occurred upstream from test station.

⁴Fish removed from boxes and transplanted to the Mackay Fish Hatchery. Those fish unaccounted for are presumed to have decomposed between inspections.

Idaho Fish and Game Department data show a rapid and increasing mortality pattern, but starting after the termination of the Forest Service testing period. The data concerning Department rainbow fingerlings show a significant mortality beginning on July 21, increasing through the latter part of July, and then diminishing in August.

Table 13 indicates that most adult rainbow losses occurred shortly after being placed in the live-boxes. Rainbow placed in the artificial stream channel on the lower North Fork

Table 13. Adult rainbow trout mortality in live-box tests by the Idaho Fish and Game Department¹

	Conti	rol stream				Test str	eams			
Inspection Date (1964)	Ge	eertson	Pai	nther	Ir	dian	Upper	No. Fork end side annel	Lower	No. Fork end side annel
	Live fish	Dead fish removed	Live fish	Dead fish removed	Live fish	Dead fish removed	Live fish	Dead fish removed	Live fish	Dead fish removed
6-29					70					
6-30			70		30 ²					
7-2			30 ²							
7-4			3		3		57			
7-5			20	10	25	5				
7-6	30						3		3	
7-8	28	2	19	1	19	6				
7-12			16	3	19					
<i>7</i> -15	25	3								
7-21			16		19					
7-22	25									
7-27	25		16		19		27 ⁴		30	
8-3	25		16		19		27			
8-6			16		19		27			
8-19										
8-21			16		19		27			
8-24			16		19		27			
9-8			16		19					
9-14	25		16		19		27		27 ⁵	
9-24	25		16		19		27		27	
10-1	25				19		27		24	3
10-2			16							
10-3									22 ⁵	
10-7	25		16		19		27		22	
10-14	25		16		19		27		22	
10-20		ŀ								
10-21	25		166		19 ⁶		27 ⁶		226	
Percent loss		17		47		37		0		7

¹From long-range study being conducted cooperatively between the Forest Service and Idaho Fish and Game Department.

²Removed weaker fish and started test period.

³First day spraying occurred upstream from test station. ⁴Thirty fish removed and placed in the lower end of side channel on Lower North Fork.

⁵Fish removed for analyses. ⁶Fish removed from boxes and transplanted to the Mackay Fish Hatchery.

sustained only minimal losses. The mortality which occurred on July 5, in both the Panther Creek and Indian Creek live-boxes, occurred only 1 day after spraying began upstream. It thus appears that these early losses were probably still the result of handling and transportation.

A question arises as to why adult and fingerling rainbow held in live-boxes at the same location on Panther Creek for the same period of time did not suffer similar mortalities. Schoenthal (1963) found that mortality from DDT was greater and quicker among small fish as compared to larger ones. His work showed that a 100 percent mortality occurred among small hatchery rainbow fingerlings $(1\frac{1}{2})$ to 3 inches) when held for 48 hours in continuous concentrations of DDT, ranging from .05 to 3.0 ppm. Larger hatchery rainbow (5.1 to 10.0 inches) sustained a 66 percent mortality when subjected to 48 hours of continuous DDT concentrations, rangnig from .25 to 10.0 ppm. The surviving fish in the latter group were held for a 30-day post-test period and suffered an additional 10 percent mortality. Schoenthal concluded that a correlation existed between the size of fish and mortality.

In the final analysis of all live-box data available, it appears that most of the mortality losses sustained in live-box studies resulted from such causes as handling, transportation, drops in water level, and possibly starvation. There is a probability, however, that the fingerling rainbow held in Department live-boxes on Panther Creek died as a result of spraying in that drainage. This does not preclude the possibility that other factors may have influenced their susceptibility to DDT spray.

Wild Fish Sampling

Samples of wild fish were taken before and after spraying for analyses of possible residue levels previous to spraying and changes resulting from the spray project. Most samples were taken with the electric shocker from various streams throughout the project and from a control stream (fig. 27).

Wild fish were taken from six streams before spraying started, from seven locations immediately after spraying, and from ten streams 3 months later. As samples were collected, they were placed in plastic bags and frozen.

Fish Residue Analyses

All samples of wild fish taken with the electric shocker were used to determine residue levels of DDT and DDT metabolites. Samples of fish used in live-box studies also were taken to determine residue levels at the beginning of the testing period and any patterns resulting from spray exposure during the testing period. As indicated previously, chinook salmon fingerlings from live-box studies were wild fish. Rainbow trout used in live-box studies were obtained from the Mackay Fish Hatchery.

Wild fish collected with the electric shocker in June and July were drawn to make separate samples of the viscera and the body. If fish were to become exposed from gorging on DDT killed aquatic insects, then the stomach and stomach contents of fish taken immediatly after spraying should most likely contain higher residue levels than the body tissues. If higher, the contents of the stomach should not be included in the total body sample because portions of residues in the stomach contents would be lost in excrement and not be absorbed as part of the body content.

All fish were handled in the same manner, regardless of source. Samples were placed in individual plastic bags, frozen, and shipped to the Agricultural Research Service Laboratory. At the laboratory the following extraction, cleanup, and analytical procedures were used:

Extraction

Samples were thawed, chopped into small pieces and ground. Small fish were not ground but were put directly in the Virtis homogenizer bowl. A weighed portion of

¹Agricultural Research Service mimeographed report PCY-65-14, 1965. (On file with Entomology Research Div., ARS, Yakima, Wash.)

Figure 27. Chinook salmon, steelhead trout, and rainbow trout were taken from streams by the use of an electric shocker. Samples were taken before the spray project started, just after it was completed, and again about 3 months later. Fish samples were put in plastic bags, frozen, and sent to the ARS laboratory where they were analyzed for DDT residues.







the ground fish was put in the Virtis bowl and blended 5 minutes with 100 ml. chloroform with the Virtis homogenizer. Anhydrous sodium sulfate was added, the sample was filtered through a cotton plug overlaid with anhydrous sodium sulfate and the flask and filter were washed until a volume of 200 ml. of Chloroform was reached.

Cleanup

A 100 ml. volume of the extract was shaken 2 times with 10 ml. fuming (15-18%) sulfuric acid, removing the acid each time, and then once with 20 ml. concentrated sulfuric acid, washed with water until the sample looked clear (4-6 washings). Anhydrous sodium sulfate and Celite 545 were added, the sample shaken and filtered through a cotton plug overlaid with anhydrous sodium sulfate. The flask and funnel were rinsed twice with chloroform. The filtrate was evaporated to dryness, the sides of the flask rinsed down with chloroform and again evaporated to dryness. Distilled n-hexane was used to rinse down the side of the flask, evaporated to dryness and this step repeated. Air and a warm water bath were used for the evaporations as for the algae. The residue was then made to a definite volume with distilled n-hexane.

Analysis

Gas chromatography. Four foot glass column — 2 ft. of 5% DC200 on acid washed Chromosorb and 2 ft. of 10% QF-10065 on acid washed Chromosorb — Research Specialties — Sr 90 Detector.

Crude extractible lipids. Five ml. of the chloroform extract were put in a weighted beaker and allowed to dry overnight and weighed again in the morning. The residue remaining was called crude extractible lipids.

Recoveries

Fish . . . that were analyzed and found to be low in DDT content were used to check the recovery of DDT and its metabolities. DDT and its metabolites added to . . . fish at rates of 0.1 to 10.0 ppm gave average

recovery of 96.6% of DDE, 91.8% of combines o,p'-DDT and TDE, and 115.7% p,p'-DDT.

Detailed reports of DDT and DDT metabolities for wild fish are given in table 14 and for fish samples taken from live-box studies in table 15.

Averages of residue levels are shown in table 16.

Summaries of total DDT residues are graphically illustrated for wild fish in figure 28 and for fish samples taken from Forest Service live-box studies in figure 29.

Every sample of fish from before spraying started, except the chinook salmon from control live-boxes, had measurable amounts of DDT residues.

Wild fish collected by shocking before spraying started contained residue levels of from .035 to .410 ppm of DDT. Some of these fish were more than a year old so that exposure to DDT could either have originated a year or more previous to this project, or DDT could have accumulated for more than a year.

Chinook fingerlings taken from the Lemhi and Pahsimeroi Rivers for live-box studies contained from less than .005 to .029 ppm DDT. Rainbow trout used in live-boxes came from the State Hatchery and contained from .055 to .234 ppm total DDT. It is important to note that these fingerlings of both species were less than 1 year old so that contamination would have resulted primarily from overwinter and spring exposure. Wild fish, including the chinook salmon from the live-box tests could only have been exposed in natural environment of native waters. This is not true for rainbow trout used in the live-box tests.

There was an aerial DDT spray project in 1956 over some of the same forested areas that were treated in this 1964 project. It is impossible to know if the prespray residue levels resulted from the 1956 project.

In live-box testing there was no increase in the residue levels of rainbow trout held as controls but there was an increase from .005 to .021 ppm in chinook salmon between the prespray and postspray period. Control fish in Haynes Creek were held in portions of the stream well above agricultural development so chances of contamination from such a source were practically nil. There was a possibility that highly volatized materials from the spray project could have drifted beyond the project boundaries. Settling out could have been at a very low rate, but possibly enough for subtle low-level exposure.

In the sampling period immediately following spraying, residue levels rose sharply in both species in live-boxes from Panther Creek

Table 14. DDT and DDT metabolites in wild fish¹ (in parts per million)

Sample No.	Location	Sample type	Date (1964)	Percent lipids	DDE	o,p'-DDT and TDE	p,p'-DDT	Total DDT
57 58 59	Colson Cr.	Rainbow Rainbow 5 rainbow	6-25 7-27 10-20	.7 1.5 5.1	.019 .272 .400	<.005 .048 .033	.016 .608 .376	.035 .928 .809
60 61 62 63	Dahlonega Cr.	Rainbow Rainbow Dolly varden 5 rainbow 1 sculpin 1 brook	6-18 7-27 7-27 10-20	1.4 1.3 2.2 3.1	.030 .144 .344 .118	.006 .048 .056 .028	.032 .736 .560 .444	.068 .928 .960 .590
72 73 74 75	Indian Cr.	Rainbow Rainbow 11 chinook	6-23 7-27 10-20 10-20	2.3 3.0 3.5 4.6	.070 .208 .660 .600	.007 .080 .076 .086	.051 .816 .760 .668	.128 1.104 1.50 1.35
76 77 78	Musgrove Cr.	Rainbow Rainbow Rainbow	6-25 7-27 10-19	1.6 2.9 4.2	.020 .290 .476	<.005 .040 .040	<.005 .490 .580	.020 .820 1.10
89 90 91	Spring Cr.	Rainbow Rainbow 7 rainbow	6-23 7-27 10-20	1.3 1.5 2.5	.032 .076 .456	<.005 .032 .060	.009 .368 .624	.041 .476 1.14
92 93	Squaw Cr.	Rainbow Rainbow	6-23 7-27	2.4 2.3	.220 .360	.020 .040	.170 .510	.410 .910
86	Panther-Napias	Fish	10-19	1.2	.860	.040	1.18	2.08
106 107 108	Lower N. Fk.	1 rainbow 2 rainbow 1 chinook	10-20 10-20 10-20	1.2 8.0 4.2	.430 .203 .041	.040 .053 <.005	.560 .763 .446	1.030 1.019 .487
101	Main Salmon	1 cutthroat	10-20	1.6	.120	<.005	.152	.272
Controls: 97 99	: Lemhi River	Rainbow 1 rainbow 1 whitefish 1 sculpin	7-19 10-20	4.6 4.9	.006 .010	.006 <.005	.010 .048	.022 .058
98 100	Lemhi River	Chinook Chinook	7-19 10-20	4.9 6.9	.005 .013	.005	.005	.100

¹ Agricultural Research Service mimeographed report PCY-65-14, 1965. (On file with Entomology Research Div., ARS, Yakima, Wash.)

and to a far lesser degree in fish from the North Fork of the Salmon. No other analysis made during monitoring could verify the increases in these streams.

It should be noted that residues in flesh are accumulations, and in these cases could be accumulations from low levels of exposure in the water, from food material, or both. It is a factor that Panther Creek has a large drainage area. In addition, the Panther Creek drainage received many days of spraying.

There was a possibility of repeated low-grade contamination providing for possible high accumulated total residue in the aquatic life. Some contributions to Panther Creek of DDT were also made by the emergency load jettisoned from the PB4Y2 plane as discussed later.

Increases in residue levels of live-box fish did occur as a result of project exposure. Mortality rates did not indicate that postspray residue levels of from .369 to 2.52 ppm

Table 15. DDT and DDT metabolites in chinook salmon and fingerling rainbow trout from live-box studies¹ (in parts per million)

Sample No.	Species	Location	Date (1964)	Percent lipids	DDE	o,p'-DDT and TDE	p,p'-DDT	Total DDT
Forest Se	ervice:							
68	Rainbow	Haynes Cr. ²	7-1	5.0	.028	.006	.021	.055
69	"		7-21	1.9	.008	<.005	.009	.017
79 80	"	Panther Cr.	7-5 7-21	2.2 1.6	.030 .207	.040 .096	.164 2.22	.234 2.52
102	и	North Fork	7-5	3.3	.007	.013	.037	.057
103	и		7-21	2.3	.038	.051	.280	.369
70	Chinook	Haynes Cr. ²	7-1	4.2	<.005	<.005	<.005	<.005
71	"		7-21	5.7	.005	.007	.009	.021
87	n	Panther Cr.	7-5	3.7	.006	.006	.011	.023
88	n		7-21	3.5	.060	.160	1.44	1.66
104	n	North Fork	7-5	4.8	.005	.008	.016	.029
105	n		7-21	1.4	.044	.029	.459	.532
Idaho Fis	sh & Game D	epartment:						
30 16 49 50	Rainbow " "	Panther Cr. """ """	7-21 7-27 8-6 8-21	.6 .4 .8 .5	.640 .484 .888 .869	.320 .340 .328 .069	1.08 .680 .700 .521	2.04 1.504 1.916 1.459
24 32 33 34	11 11 11	Indian Cr. """ """	7-4 10-1 10-7 10-14	5.7 .6 .8 .6	.028 .278 .313 .618	.005 <.005 .007 <.005	.021 .074 .097 .046	.054 .352 .417 .214
23	n	Geertson Cr. ²	7-6	4.9	.031	.006	.015	.052
52	n		10-14	1.2	.070	<.005	.021	.091

¹Agricultural Research Service mimeographed report PCY-65-14, 1965. (On file with Entomology Research Div., ARS, Yakima, Wash.)

²Control streams.

were up to the acute lethal range. The ultimate physiological results of these sublethal levels cannot be predicted from any information gathered in these studies.

Wild fish had nearly the same trend in residue levels. There was a sharp rise from prespray to postspray levels for all six creeks sampled in the project area. In three of five streams the increase continued from July to October. Prespray and July samples were not taken in Panther Creek, the North Fork of Salmon River, nor the Salmon River. October residue levels for the North Fork and main Salmon samples were comparable to other samples, whereas the Panther Creek sample was higher than at any other sampling location. Similar reasoning may be applied for wild fish results on Panther Creek as has been indicated for live-box fish, i.e., that no other measurement in this monitoring program indicated exposures which could have produced these accumulated levels. Exposure must have been lingering or persistent for levels to have continued to increase at three stations for 3 months after the project.

Residue levels for the viscera in those instances where it was drawn and made a separate sample are presented in table 17.

Comparing residue levels of viscera with residue levels of corresponding body samples (table 14) there appears to be no constant difference. Residue levels in viscera are sometimes slightly less than and sometimes slightly more than those in the body. June and July figures in table 14 are of body without viscera. Had the viscera been included, the results would not have changed significantly.

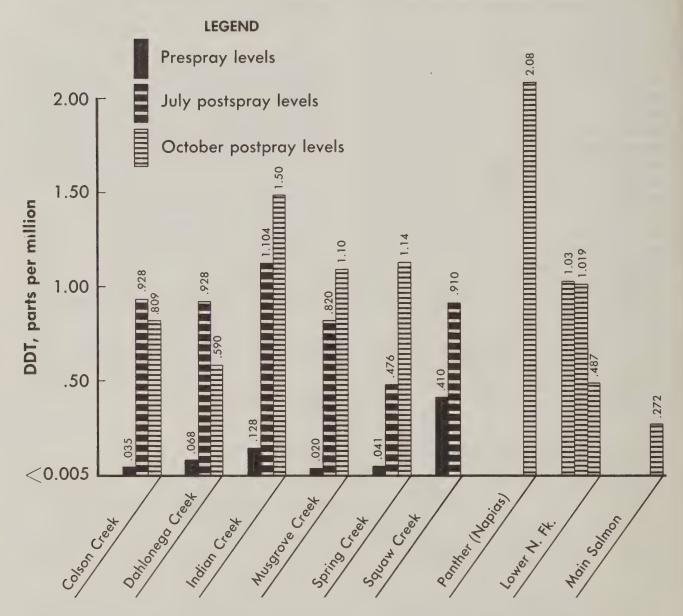
As a part of the analyses of fish made for this project, extracts were made of crude lipids. (Lipids are any of a group of substances comprising fats and other esters that possess similar properties.) The precent of lipids reported does not represent all the lipids present in the sample. The percent of lipids reported represents a uniform portion of the total lipid content of the tissue and as such can be used as a basis of comparison between samples of the same types of tissues from the same species.

Table 16. Average DDT residue levels in fish (parts per million)

Wild fish:		Prespray	Postspray					
		June	July	August	October			
Project streams — Average		.117	.875		1.037			
	Low-high	.020410	.476 - 1.104		.272 - 2.08			
Controls	Average		.019		.079			
	Low-high		.015022		.058158			

Live-box rainbow:		Prespray	Postspray					
			July	August	October			
Project streams — Average		.115	1.608	1.687	.328			
	Low-high	.054234	.369 - 2.52	1.459 - 1.916	.214417			
Controls	Average	.054	.017		.091			
	Low-high	.052055	(one sample)		(one sample)			

Figure 28. Total DDT residues found in wild fish in parts per million.



Information on extractable lipids is presented in table 18. The significance of these lipid levels to fish physiology is not within the scope of this project. Patterns of trend in levels might be and hence, are presented here. Lipid contents decreased from June to July in all samples of live-box fish except the chinook salmon held at the control station. The reverse is true for all wild fish sampled except from Squaw Creek and Dahlonega Creek, which showed only negligible decreases. Increases continued for all wild fish samples from the project area for the October sampling.

Differences in live-box held fish and wild fish could be expected because of the differences in feeding — live-box fish being at least partially isolated from normal food availability by the artificial habitat whereas wild fish could make use of the summer insect hatch and production.

Information from table 18 should be compared to information in tables 10 and 12. Rainbow at all three live-box stations operated by the Forest Service show a lipid content of 1.6 percent or more in the July postspray period which was the end of the testing

Table 17. DDT residue levels (in parts per million) of viscera of wild fish¹

Sample number ²	Location	Sample type	Date (1964)	Percent lipid	DDE	o,p'-DDT and TDE	p,p'-DDT	Total DDT
57a	Colson Cr.	Rainbow	6-25	1.4	.021	<.005	.005	.026
58a	" "	"	7-27	2.4	.276	.060	.160	.496
60a	Dahlonega Cr.	"	6-18	3.1	.040	<.005	.010	.050
61a	" "	"	7-27	4.2	.200	.140	1.20	1.540
62a	" "	Dolly varden	7-27	3.2	.360	<.005	.240	.600
72a	Indian Cr.	Rainbow	6-23	3.3	.080	.009	.015	.104
73a	" "	"	7-27	3.8	.400	.096	.520	1.016
76a	Musgrove Cr.	"	6-25	2.1	.048	.017	<.005	.065
77a	11 11	"	7-27	5.3	.176	.072	.384	.632
89a	Spring Cr.	n .	6-23	2.4	.028	<.005	.005	.033
90a	" "	"	7-27	3.5	.244	.115	.316	.675
92a	Squaw Cr.	"	6-23	3.3	.200	.024	.041	.265
93a	" "	"	7-27	3.7	.630	.146	.600	1.376

¹Agricultural Research Service mimeographed report PCY-65-14, 1965. (On file with Entomology Research Div., ARS, Yakima, Wash.)

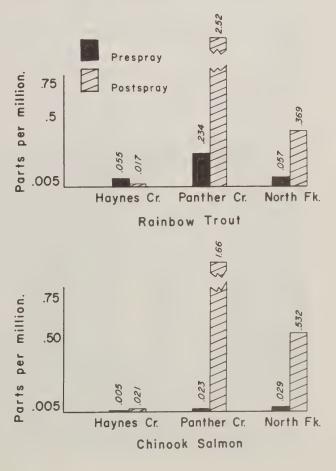
²The letter a designates the corresponding sample number as shown in table 14.

period. Those from the Idaho Fish and Game Department live-box in Panther Creek had 0.6 percent from the July 21 samples and 0.4 percent for the July 27 samples. Fish taken on the earlier date were of the beginning of the heavy losses which occurred in that livebox. Those sampled in August still had less than 1 percent lipid content. The Indian Creek prespray sample contained 5.7 percent lipid content, but when the fish mortality started to occur on October 1, lipid contents were below 1 percent. The prespray control sample from Geertson Creek also contained a high lipid content. By October 14, the contents had not dropped to below 1 percent and small losses were just starting to occur.

There are a number of questions regarding lipid content which may be asked but not answered in this study:

1. Is there a threshold of survival relative to lipid content?

Figure 29. Total DDT residues found in rainbow trout and chinook salmon from Forest Service live-box studies.



- 2. Does DDT exposure affect this threshold?
- 3. Are fish more susceptible to DDT when lipid contents are low, for instance below 1 percent?
- 4. Are fish more susceptible to DDT when
- building up lipid content or loosing it?

 5. If either one of the last is true over the other, then do fish in live-box testing where they lose lipid contents have the same DDT susceptibility of wild fish which are gaining in lipid content?

Table 18. Extractable crude lipids of fish sampled for DDT residues¹

			Percent extr	actable lipids	
Location	Sample type	Prespray	July postspray	August postspray	October postspray
Live-box fish:					
Forest Service:					
Haynes Cr. ²	Rainbow	5.0	1.9		
Panther Cr	Rainbow	2.2	1.6		
No. Fork Salmon	Rainbow	3.3	2.3		
Average	Rainbow	3.50	1.93		
Haynes Cr. ²	Chinook	4.2	5.1		
Panther Cr	Chinook	3.7	3.5		
No. Fork Salmon	Chinook	4.8	1.4		
Average	Chinook	4.23	3.33		
Idaho Fish and Game Depa	rtment:				
Panther Cr	Rainbow		.6 & .4	.8 & .5	
ndian Cr	Rainbow	5.7			.6 & .8
Geertson Cr. ²	Rainbow	4.9			1.2
Average	Rainbow	5.30	.50	.65	.87
Wild fish:					
Forest Service:					
Colson Cr	Rainbow	0.7	1.5		5.1
Dahlonega Cr	Rainbow	1.4	1.3		3.1
ndian Cr	Rainbow	2.3	3.0		3.5
Musgrove Cr	Rainbow	1.6	2.9		4.2
Spring Cr	Rainbow	1.3	1.5		2.5
Squaw Cr	Rainbow	2.4	2.3		
Panther (Napias)					1.2
ower No. Fork	Rainbow				1.2
	Rainbow				8.0
Main Salmon					1.6
Lemhi River ²	Rainbow		4.6		2.7
emhi River ²	Chinook		4.9		6.9
Average	Rainbow	1.62	2.75		3.64

¹ Agricultural Research Service mimeographed report PCY-65-14, 1965. (On file with Entomology Research Div., ARS, Yakima, Wash.)

² Control streams.

Aquatic Vegetation

The following quote is from Jensen and Gaufin (1964b):

Many insecticides are concentrated in aquatic plants to many times their original application levels. Hoffman and Drooz (1953) reported that chemical analyses of moss collected from a small stream 1 month after an aerial application of DDT revealed concentrations of 0, 44, 110, and 128 ppm of DDT in the moss at distances of 1, 2, 3, and 6 miles below the source of the stream. It appeared that DDT particles accumulated on stream plants in considerable amounts downstream, coinciding with insect mortality which also increased from the source to the mouth of the stream.

To examine prespray DDT residue levels and increases that might occur following the spraying program, prespray and postspray (October) samples of aquatic vegetation were collected from 10 sites (fig. 24). Emphasis was given to collecting samples from streams important as salmon and steelhead spawning and rearing grounds. Both emergent (Carex spp., Equisetum spp. and mosses) and submerged type (slime algae) aquatic vegetation were collected.

Samples were put in plastic bags, labeled, frozen, and shipped to the Agricultural Research Service Laboratory for analysis.

Extraction and cleanup procedures were as follows: (Analysis was the same as described for fish.)

Extraction

A weighed portion of the sample was blended 3 minutes with chloroform at the ratio of 2 ml. of chloroform to 1 gram of sample. Anhydrous sodium sulfate was added and the sample was filtered through a cotton plug containing anhydrous sodium sulfate.

Cleanup

An aliquot of the sample was evaporated

¹Agricultural Research Service mimeographed report PCY-65-14, 1965. (On file with Entomology Research Div., ARS, Yakima, Wash.)

with air in a warm water bath to approximately 25 ml. One and one-half grams of a mixture of Attaclay: magnesium oxide and Nuchar C-190-N $(4:3:1\frac{1}{2})$ was added and the sample shaken for 1 minute and filtered through a cotton plug overlaid with anhydrous sodium sulfate, with the flask and filter being washed twice with chloroform. The filtrate was then evaporated to dryness in a warm water bath using a gentle stream of air. The sides of the flask were washed down with chloroform and the evaporation was repeated. The sides of the flask were then rinsed with distilled n-hexane and the evaporation repeated. The residue was then taken up in a definite volume of distilled n-hexane.

There was not a surplus of aquatic vegetation to use for determining recovery rates.

Table 19 shows results of analyses for DDT residues in the aquatic vegetation. Data are divided according to the two types of vegetation. Of interest is the fact that of prespray samples one of the three emergent samples and four of the 10 submergent samples had measurable amounts of DDT. Every postspray

Figure 30. Samples of both submergent and emergent aquatic vegetation were collected before and after spraying to analyze for DDT residues.



Table 19. DDT content of aquatic vegetation¹

				Residues in parts per million ²			
Sample number	Stream	Date (1964)	DDE	o,p'-DDT and TDE	p,p'-DDT	Total DDT	
Submergent	vegetation:			4.			
121 122	Boulder Boulder	7-5 10-22	<.005 .009	<.005 .027	.034 .107	.034 .143	
123	Colson	6-25	<.005	<.005	<.005	<.005	
126 127	Dahlonega Dahlonega	6-19 10-20	<.005 .020	<.005 .040	<.005 .090	<.005 .150	
132 133 135	Indian Indian Indian	6-23 6-23 10-20	<.005 <.005 .008	<.005 <.005 .013	<.005 .005 .044	<.005 .005 .065	
140 141	Moose Moose	6-18 10-24	<.005 .022	.007 .040	.014 .324	.021 .386	
142 143 144	Musgrove Musgrove Musgrove	6-25 6-25 10-19	<.005 <.005 <.005	<.005 <.005 .008	.007 <.005 .046	.007 <.005 .054	
138	No. Fork Salmon	10-20	<.005	<.005	.017	.017	
150 151	Spring Spring	6-23 10-20	<.005 <.005	<.005 .008	<.005 .014	<.005 .022	
1 <i>5</i> 3 1 <i>5</i> 4	Wagonhammer Wagonhammer	6-26 10-24	<.005 <.005	<.005 <.005	<.005 .040	<.005 .040	
137 136	Lemhi ³ Lemhi	7-19 10-20	<.005 <.005	<.005 <.005	.007 <.005	.007 <.005	
Emergent ve	egetation:						
124	Colson	6-25	.005	<.005	<.005	.005	
125	Dahlonega	6-19	<.005	<.005	<.005	<.005	
134	Indian	6-23	<.005	<.005	<.005	<.005	
139	Main Salmon	10-29	.009	.010	.031	.050	
136	Lemhi ³	10-20	<.005	<.005	<.005	<.005	
Averages Submergent				Tota Prespray .007	Postspray .109		
Emergent:	Control streams Project streams Control streams			.007 ⁴ .002	<.005 ⁴ .050 ⁴ <.005 ⁴		

¹Agricultural Research Service mimeographed report PCY-65-14, 1965. (On file with Entomology Research Div., ARS, Yakima, Wash.)

².005 ppm — lower limit of sensitivity of method used.

³Control stream.

⁴Only one sample.

sample contained DDT, evidencing that aquatic vegetation does hold DDT although the levels found were quite low. Recognizing that the postspray samples were taken 3 months after the spray project, the low levels found do not seem to indicate any particularly strong affinity whereby aquatic vegetation gathered and held DDT residues.

DISCUSSION

Conditions of terrain and weather, size and duration of this project, as well as the intricate network of streams requiring protection, exacted a strenuous test of the protection program — a program designed to minimize impacts on an aquatic environment and yet allow maximum treatment of the spruce budworm.

Evaluations indicated that under conditions existing at this project, helicopters, with experienced pilots, can maintain satisfactory control of the spray material used. A minimum of 400 feet must be left between the prism of planned spray application and the protection objective, however. This is a minimum distance and does not allow room for deviations from spray control specifications.

Planes of the TBM class or larger require a greater margin of protection. Of those spray planes used, the TBM class was most effective in maintaining application control but required a minimum of 1,400 feet between the application prism and the stream. Again, this is a minimum distance and does not allow for errors.

Multi-engine planes did not have adequate control of spray materials to provide the required protection from DDT drift when attempting to observe a 1,400 foot protection zone. The minimum width of protection zone needed for consistent protection was not determined for the multi-engine planes.

The indicated protection widths are only adequate so long as constant care and surveillance are maintained. Differences of a few hundred feet in what constitutes protection zone widths mean very little if laxity occurs anytime during the operations. Other protec-

tive measures must also receive strict compliance. Weather conditions which might affect spray distribution are so critical that localized situations occur which are not detected in the general weather observation and forecasting program. Such difficulties were experienced on this project. This was especially true because of the steep terrain and the sharp variations in exposure and ground cover existent in the project area.

All protection phases of a spray program must be constantly observed during the entire course of any application project. Pressures on operational and contract personnel establish the principal objective as job completion with satisfactory target insect control. Protection objectives are easily slighted.

Evaluation of possible spraying impacts on the aquatic environment was designed to measure spray materials which might contaminate the streams, the impact on the stream fauna and flora, and immediate impacts on fish.

Problems of determining the occurrence, intensity, and duration of contaminants in free-flowing streams by sampling procedures are still not satisfactorily resolved. Dip samples are too much hit or miss. A technique of drawing off continuous samples of water by the use of a burette system was demonstrated on this project. Results showed traces of DDT in the water a number of times when not detected by dip sampling.

No water sampling indicated significant amounts of spray materials in the project streams. Only occasional extremely minute amounts were found. The system of water sampling used on this project did not divulge contamination which was not detected more thoroughly or definitely by some other monitoring procedure. Only on occasions did water sample results verify other findings.

Stream insect fauna provides the principle source of food for fish and is, therefore, of primary concern as the basis for fish production. This fauna is also extremely sensitive and demonstrates acute reactions to contaminants within its environment. Evaluations of

this aquatic life provided the best guide as to what effect spraying was having on the streams.

Fisheries personnel using the criterion of drifting aquatic insects to indicate acute impacts on streams were the first to recognize inherent weaknesses of the technique. It remains the consensus of fisheries personnel, however, that no other technique has yet been demonstrated as a satisfactory substitute for this type of evaluation. Supplemental information could be gathered to make results derived from this technique more meaningful:

- 1. More intensive studies as to the meaning of drifting aquatic insects are needed. It is not certain that all drifting aquatic insects should be considered as inevitably dead. This question exists whether it is DDT or any other stimulus in the water. If some drifting insects remain alive, how many, and can a correction factor then be applied to the drift sampling results?
- 2. Reasons for changes which might occur in insect populations during and after a treatment project cannot be positively identified without knowledge of normal seasonal fluctuations.
- 3. If any or all drifting aquatic insects are losses from the stream bottom population, what does it mean to the food source for fish production? Will the losses actually impose a limiting factor in the maintainance of an otherwise normal fisheries resource?

Sampling stream bottom fauna still can provide much of the basic information needed as to the impact of spraying on the fish food production. There were wide variations between streams in the prespray bottom samples, in the postspray samples, and in the type of changes which took place. Cyclic fluctuations in numbers, volume, and composition of bottom fauna are normal in a stream. Refinement of the bottom sampling technique used on this project would be necessary in another project to stratify as many of the normal variables as possible. Impacts from an insecticide treatment could then be more accurately identified.

Differences in stream ecotypes results in differences in the production of bottom fauna. Without complete stratification of all measurable variables it is unreliable to make a broad spectrum comparison between any stream in a treatment area and a control stream. It would be much better to predetermine the normal fluctuations occurring in the treatment stream for use as a comparison after spraying. The determination should be made at least one comparable season before treatment.

Bottom sampling indicated no instance of complete bottom fauna annihilation. In fact, there was a sharp reduction in bottom insect numbers at only one station, the Upper North Fork. At that station, there was no marked recuperation within 3 months following spraying. This individual situation correlated closely with some of the heaviest drifting insect numbers and with one of three series of water samples containing measurable amounts of DDT. Many stations had significant increases in total numbers of insects within months after spraying. This indicated excellent recruitment although species composition may have been altered from the norm for an untreated population.

If aquatic vegetation does absorb DDT and keeps it longer, it did not show up strongly on this project. Sampling did indicate that residues were picked up by the vegetation. But either vegetation does not have a strong affinity or the amounts of DDT in the water were too minute for abnormal accumulations.

Live-box testing of fish pointed up some interesting observations, although none disclosed acute toxicity directly attributable to DDT contamination. It is almost impossible to obtain fish, either hatchery reared or wild, which do not have detectable levels of DDT in their body tissue before being introduced as test subjects. In spite of precautions used and the fact that detection techniques indicated only occasional small amounts of spray materials had entered streams, there was an increase of residue levels between pre- and postspray periods in all fish sampled within the project area. This occurred in all but one control sample. Thus, DDT did get into the

streams and into fish by subtle means which were not detectable by the monitoring measures.

The highest residue level found in any fish within the regular project area was 2.52 ppm and only three samples had more than 2.0 ppm. No interpretations can be made as to what these levels mean except that they appear to be sublethal as to acute toxicity levels.

An observation pertaining to the physiological welfare of fish resulted from the fish analysis and deserves further exploration. All or most DDT residues are found in the lipid contents of fish flesh. A crude lipid extraction was made of all samples analyzed. Wild fish registered relatively low lipid contents at the beginning of the summer season. Sampling indicated an increase through the summer, however. Conversely, hatchery reared fish

registered the highest lipid contents when taken from the hatchery and lost lipids as they were held in live-boxes. The question arises as to the DDT tolerances and absorption rates which might result from these two physiological conditions.

Monitoring of this project was intended to partially measure resultant levels of DDT residues and to help minimize the impacts which spraying might have on the aquatic ecosystem. To this goal, the monitoring was successful. Total elimination of impact on stream environs seems impossible for any DDT project which is effective in controlling the target insect. Administrative decision to use DDT in other projects of this type should rely on results of research which identifies what sublethal levels of residues mean to fish, fish productivity, and fish habitat.

TERRESTRIAL MONITORING

Several species of big game and birds are hunted within the Salmon National Forest. Hunting is important to the autumn economy of Salmon and smaller communities within or near the Forest. This resource warrants attention and evaluation for possible impacts which might influence wildlife populations, hunting, economies resulting from hunting, or the general well-being of bird and mammal species.

Experiences elsewhere in aerial spray applications of DDT at rates used in this project show no acute losses of birds or mammals. Possible long-range implications from sublethal exposures to pesticides are of increasing concern to wildlife conservationists and managers. Potential hazards to human health for those who depend on wild game for much of their protein diet are of concern to the users and to wildlife managers.

Toxicogenic effects of DDT and DDT metabolites on birds and animals are not well known. Investigation in this area requires research techniques which cannot be applied under field conditions. Related studies are being carried on under controlled conditions at various research locations. When results are obtained, they may be used to interpret some field observations.

Project monitoring did not delve into research of long-range physiological effects from sublethal exposures to pesticides on birds or big game. Monitoring was designed primarily to survey resultant levels of DDT residues in the fatty tissues and flesh of birds and animals. It did not attempt to evaluate intricate ecological changes resulting from the spruce budworm infestation or from programs to control the infestation.

BIG GAME Collections

Mule deer (Odocoileus hemionus) and elk (Cervus canadensis) were the primary forest

big-game species subjected to exposure and were thus scheduled for sampling. Mountain sheep (Ovis canadensis), which spend much of their time in timber types, were programmed for sampling, but without success. Mountain goats (Oreamnos americanus) frequent the alpine and subalpine zones in the upper portions or above forest types. This species was selected for sampling to explore the possibility that volatile materials from aerial spraying might drift upward and be deposited at higher elevations.

For comparative purposes, big-game animals of these game species were sampled in other areas. Information from previous sampling in Idaho and from sampling in other sprayed and nonsprayed areas has also been summarized for comparison purposes.

Because DDT is long-lived, residues in aerial portions of vegetation may be expected to persist and be a source of exposure to foraging animals. In fact, the residues may persist a year or more on aerial portions of perennial vegetation, thus providing exposure sources to animals using it in future years. This source of exposure could be available to animals born in years subsequent to spraying. Residues may be transmitted to the young of animals through the mother's milk. Also, it is possible for residues to be transmitted from mother to young during fetal development.

To explore some effects of these delayed exposures, adipose tissue samples will be taken from mule deer shot during the hunting seasons of 1965 and 1966. Special efforts will be made to sample fawns and yearlings for each of these years.

DDT is oil or fat soluble and is stored primarily in the lipids of tissues. The adipose or fatty tissues were, therefore, sampled to evaluate accumulated residue levels. Samples of liver, kidney, thyroid, and adrenal tissues from some of the mule deer also were taken for analyzing. Residues found in these tissues can be expected to be contained primarily in the lipid contents.

Residue analysis results are normally reported as parts per million of total sample tissue weight. Such analyses do not reflect total body DDT content and cannot even be directly compared to similar tissue analyses either from the same organism or from other organisms.

No tissues contain 100 percent lipids. There are also different types of lipids which can tie up different amounts or different metabolites of DDT in different ways. Variation in the qualitative composition of lipids between species may be so great that a comparison of extractable lipid amounts and DDT residue amounts between species cannot legitimately be made. Similarities of the qualitative composition might be expected between lipids of different animals of the same species.

Assuming that all DDT residues are tied up in the lipid contents, a comparison of DDT residues within a species seems legitimate provided the results are standardized to lipid content base rather than tissue content.

This can be done by the formula:

$$\begin{bmatrix} \text{Residue level} \\ \text{based on} \\ \text{extractable} \\ \text{lipids} \end{bmatrix} = \begin{bmatrix} \text{Residue} \\ \text{level} \\ \text{of tissue} \end{bmatrix} \mathbf{x} \begin{bmatrix} \underline{100} \\ \text{percent} \\ \text{extractable} \\ \text{lipids} \end{bmatrix}$$

A tissue with 100 ppm DDT content and 50 percent extractable lipids has the same lipid residue level as a tissue having 10 ppm DDT content and only 5 percent extractable lipids.

A list of big game collections planned and made appears in table 20. Individual animals taken are listed in table 21 and locations where exposed animals were taken are indicated in figure 31.

Five mule deer were killed as controls within the Panther Creek drainage prior to spray operations. Nine were taken from the same general locale approximately 1 month after the spray project. These 14 animals were shot so that a number of tissues could be sampled for analyses. Capture of additional

Table 20. Species, numbers, and purposes of big-game animals sampled for DDT residue analyses

			Number of		Samples collected					
Species	Area	Purpose	samples	Number	Dates (1964) ¹					
			in plans	taken	From	То	Sample numbers			
Mule deer	Panther Creek	Controls	5	5	6-23	6-30	1-52			
Mule deer	Panther Creek	1 mo. postspray	10	9	8-20	8-23	6-142			
Mule deer	Project area	3 mos. postspray	20	16	9-27	10-31	1 <i>5</i> -18, 32, 37-46, 51			
Mule deer	Boise & Elmore Counties	Controls	20	28	10-10	12-12	71, 72, 74-99			
Mule deer	Project area	15 mos. postspray	20							
Mule deer	Project area	27 mos. postspray	20							
Elk	Project area	3 mos. postspray	10	13	9-26	11-5	31, 33-36, 47-50, 53, 55, 115, 116			
Elk	Payette River	Controls	10	2		11-13	70, 73			
Mt. goat	Horse Creek	3 mos. postspray	6	5	8-31	9-6	57-61			
Mt. goat	Pahsimeroi	Controls	10	10	11-28	12-5	19, 20, 22-29			
Mt. sheep	Project area	3 mos. postspray	5	0						

¹Two date exceptions are the 15 and 27 months postspray mule deer which are 1965 and 1966 respectively.
²Rumen samples and samples of liver, kidney, adrenal, and thyroid tissues taken from animals numbered 2 through 10. Adipose tissue samples taken from these animals and all others listed in this table.

Table 21. Big-game animals sampled for residue analyses

Sample number	Species ¹	Sex	Age	Date collected (1964)	Location			
			Prespray s	amples from s	spray area			
1	Deer	М	5 1/2	6-23	Musgrove Cr., approx. 1/4 mile above With ington cabin			
2	Deer	М	4	6-23	1/4 mile south from mouth of Cabin Cr.			
3	Deer	М	10+	6-24	Mouth of Musgrove Cr. off Panther Cr. road			
4	Deer	М	6+	6-30	3 miles up Meyers Cove road from mouth (Rams Cr. (on Silver Cr.)			
5	Deer	М	4	6-30	3 miles up Meyers Cove road from mouth a Rams Cr.			
		One	e-month posts	pray samples	from spray area			
6	Deer	М	6+	8-20	5 miles up Porphyry Cr. road from junctic with Panther Cr.			
7	Deer	М	4	8-20	Same as No. 6			
8	Deer	М	1	8-20	South bank of Panther Cr., 500 yards stream from junction with Moyer Cr.			
9	Deer	W	2	8-20	1 mile up S. Fk. Fawn Cr. road from its juition with Copper Cr.			
10	Deer	W	2	8-21	Upper Copper Cr., 1 mile from Copper-K mine on Deer Cr. road			
11	Deer	М	1 1/2	8-22	Near mouth of Porphyry Cr. on north ridge			
12	Deer	М	4 1/2	8-22	Ridge north of Copper Cr. along road frawn Cr. ½ mile south of sample 9			
13	Deer	М	1 1/2	8-22	Ridge north of Copper Cr. along road			
14	Deer	М	2 1/2	8-23	Moyer Cr., north ridge			
		Huntii	ng season sar	mples collecte	ed from spray area			
15	Deer	F	Yearling	9-27	Red Rock Lookout—Panther Cr.			
16	Deer	М	Mature	10-3	Meyers Cove—Camas Cr.			
17	Deer	М	Mature	10-3	Panther Cr.			
18	Deer	F	Fawn	10-3	Porphyry ridge—Panther Cr.			
31	Elk	М	Mature	9-26	Granite Mt.—N. Fk., Salmon			
32	Deer	М	Yearling	9-27	Spruce Cr.—N. Fk., Salmon			
33	Elk	М	Mature	9-26	Blackbird Cr.—Panther Cr.			
34	Elk		Mature	9-26	Otter Cr.—Panther Cr.			
35	Elk	F	Mature	9-26	Moyer Cr.—Panther Cr.			
36	Elk	F	Mature	10-17	Indian Cr.—below N. Fk., Salmon			
37	Deer	М	Yearling	10-12	Nez Perce Cr.—N. Fk., Salmon			
38	Deer	F	Mature	10-11	Nez Perce Cr.—N. Fk., Salmon			
39	Deer	М	Yearling	10-11	Humbug Cr.—N. Fk., Salmon			
40	Deer	М	Mature	10-12	Sage Cr.—below N. Fk., Salmon			
41	Deer	M	Mature	10-11	Sheep Cr.—N. Fk., Salmon			
42	Deer	M	Fawn	10-25	Boyle Cr.—above N. Fk., Salmon			

Table 21. (continued)

Sample number	Species ¹	Sex	Age	Date collected (1964)	Location
43	Deer	М	Mature	10-11	Napias Cr.—Panther Cr.
44	Deer	F	Mature	10-17	Brushy Gulch—below N. Fk., Salmon
45	Deer	M	Mature	10-17	Sage Cr.—below N. Fk., Salmon
46	Deer	М	Mature	10-18	Hughes Cr.—N. Fk., Salmon
47	Elk	М	Mature	10-18	Bear Basin—below N. Fk., Salmon
48	Elk	М	Mature	11-5	Sheep Cr.—N. Fk., Salmon
49	Elk	F	Mature	10-26	Sheep Cr.—N. Fk., Salmon
50	Elk	F	Mature	10-28	Sheep Cr.—N. Fk., Salmon
51	Deer	M	Fawn	10-31	Sheep Cr.—N. Fk., Salmon
53	Elk		Mature	10-29	Sheep Cr.—N. Fk., Salmon
55	Elk		Yearling	11-4	Sheep Cr.—N. Fk., Salmon
57	Mt. goat	М	Mature	8-31	Skunk Camp—Horse Cr.
58	Mt. goat	М	Mature	8-31	Skunk Camp—Horse Cr.
59	Mt. goat	F	Mature	9-6	Skunk Camp—Horse Cr.
60	Mt. goat	М	Mature	9-5	Skunk Camp—Horse Cr.
61	Mt. goat	F	Mature	9-5	Skunk Camp—Horse Cr.
115	Elk	М	Mature	11-5	Sheep Cr.—N. Fk., Salmon
116	Elk	F	Yearling	11-2	Stein Mt.—above N. Fk., Salmon
19	Mt. goat	F	Mature	11-28	Falls Cr.—Pahsimeroi
20	Mt. goat	F	Mature	11-28	Morgan Cr.—Pahsimeroi
22	Mt. goat		Mature	11-28	Patterson Cr.—Pahsimeroi
23	Mt. goat	W	Mature	11-28	Morgan Cr.—Pahsimeroi
24	Mt. goat	W	Mature	11-28	Patterson Cr.—Pahsimeroi
25	Mt. goat	M	Mature	11-28	Patterson Cr.—Pahsimeroi
26	Mt. goat	М	Mature	11-28	Falls Cr.—Pahsimeroi
27	Mt. goat		Mature	11-29	Falls Cr.—Pahsimeroi
28	Mt. goat	М	Mature	12-4	Patterson Cr.—Pahsimeroi
29	Mt. goat	-	Mature	12-5	Big Cr.—Pahsimeroi Boise Co. Sec. 5, T10N, R9E
70	Elk	F	Mature	11-13	Boise Co. Sec. 10, 110N, R9E
<i>7</i> 1	Deer	M	Mature	12-12 12-12	Boise Co. Sec. 14, T9N, R9E
	Deer	M	Mature	12-12	
72		E	AAaatuun	11 12	ROSS CO DEC DILLUM RAE
72 73	Elk	F	Mature	11-13	Boise Co. Sec. 3, T10N, R9E
72 73 74	Elk Deer	F	Mature Mature		Boise Co. Sec. 3, T6N, R9E
72 73 74 75	Elk Deer Deer	F F	Mature	10-18	Boise Co. Sec. 3, T6N, R9E Boise Co. Sec. 20, T6N, R6E
72 73 74 75 76	Elk Deer Deer Deer	F F	Mature Fawn	10-18 10-18	Boise Co. Sec. 3, T6N, R9E Boise Co. Sec. 20, T6N, R6E Boise Co. Sec. 20, T6N, R6E
72 73 74 75 76 77	Elk Deer Deer Deer Deer	F F M	Mature Fawn Mature	10-18 10-18 10-17	Boise Co. Sec. 3, T6N, R9E Boise Co. Sec. 20, T6N, R6E Boise Co. Sec. 20, T6N, R6E Boise Co. Sec. 20, T7N, R6E
72 73 74 75 76 77 78	Elk Deer Deer Deer Deer Deer	F F M M	Mature Fawn Mature Mature	10-18 10-18 10-17 10-16	Boise Co. Sec. 3, T6N, R9E Boise Co. Sec. 20, T6N, R6E Boise Co. Sec. 20, T6N, R6E Boise Co. Sec. 20, T7N, R6E Boise Co. Sec. 35, T7N, R6E
72 73 74 75 76 77 78 79	Elk Deer Deer Deer Deer Deer Deer	F F M M	Mature Fawn Mature Mature Mature	10-18 10-18 10-17 10-16 10-17	Boise Co. Sec. 3, T6N, R9E Boise Co. Sec. 20, T6N, R6E Boise Co. Sec. 20, T6N, R6E Boise Co. Sec. 20, T7N, R6E Boise Co. Sec. 35, T7N, R6E Boise Co. Sec. 6, T6N, R7E
72 73 74 75 76 77 78	Elk Deer Deer Deer Deer Deer	F F M M	Mature Fawn Mature Mature	10-18 10-18 10-17 10-16	Boise Co. Sec. 3, T6N, R9E Boise Co. Sec. 20, T6N, R6E Boise Co. Sec. 20, T6N, R6E Boise Co. Sec. 20, T7N, R6E Boise Co. Sec. 35, T7N, R6E

Table 21. (continued)

Sample number	Species ¹	Sex	Age	Date collected (1964)	Location
83	Deer	М	Mature	Oct.	Boise Co. Sec. 32, T5N, R7E
84	Deer	М	Mature	10-13	Boise Co. Sec. 17, T7N, R4E
85	Deer	F	Mature	10-13	Boise Co. Sec. 22, T9N, R7E
86	Deer	F	Mature	10-12	Boise Co. Sec. 4, T10N, R8E
87	Deer	М	Mature	10-13	Boise Co. Sec. 24, T7N, R4E
88	Deer		Mature	10-11	Boise Co. Sec. 27, T7N, R8E
89	Deer	М	Mature	10-22	Elmore Co. Sec. 5, T5N, R8E
90	Deer	F	Mature	10-10	Elmore Co. Sec. 10, T6N, R9E
92	Deer	F	Mature	10-30	Elmore Co. Sec. 6, T3N, R4E
93	Deer	М	Mature	10-30	Elmore Co. Sec. 6, T3N, R4E
94	Deer	М	Mature	10-31	Elmore Co. Sec. 2, T3N, R6E
95	Deer	F	Mature	10-31	Elmore Co. Sec. 2, T3N, R6E
96	Deer	М	Fawn	10-31	Elmore Co. Sec. 7, T5N, R9E
97	Deer	М	Mature	12-12	Boise Co. Sec. 30, T9N, R5E
98	Deer	F	Mature	12-12	Boise Co. Sec. 14, T9N, R9E
99	Deer	F	Mature	12-12	Boise Co. Sec. 10, T10N, R9E

¹ All deer are mule deer.

animals, using a tranquilizer gun was attempted. Biopsy samples of adipose tissue were to be taken. Then the animals were to be marked, belled, and released for prospective recapture and biopsy. Repeated biopsy of the same animal would have provided a sequence in residue levels in relation to exposure. This venture failed.

Adipose Tissue Analyses

A sample of adipose tissue was taken from each animal listed in table 21. Each sample was placed in a separate vial furnished by the Agricultural Research Service, frozen, and held until collection groups were completed. The samples were then shipped to the Agricultural Research Service Laboratory. Analyses for both isomers of DDT, plus DDE and TDE were made. The results for samples 1 through 14, (table 21), were given in an

individual report. The following is a description of procedures excerpted from that report.

The samples were weighed when received in the laboratory, the weight recorded, the plastic vial sealed with masking tape and the samples frozen. The samples were kept frozen until just prior to analyses.

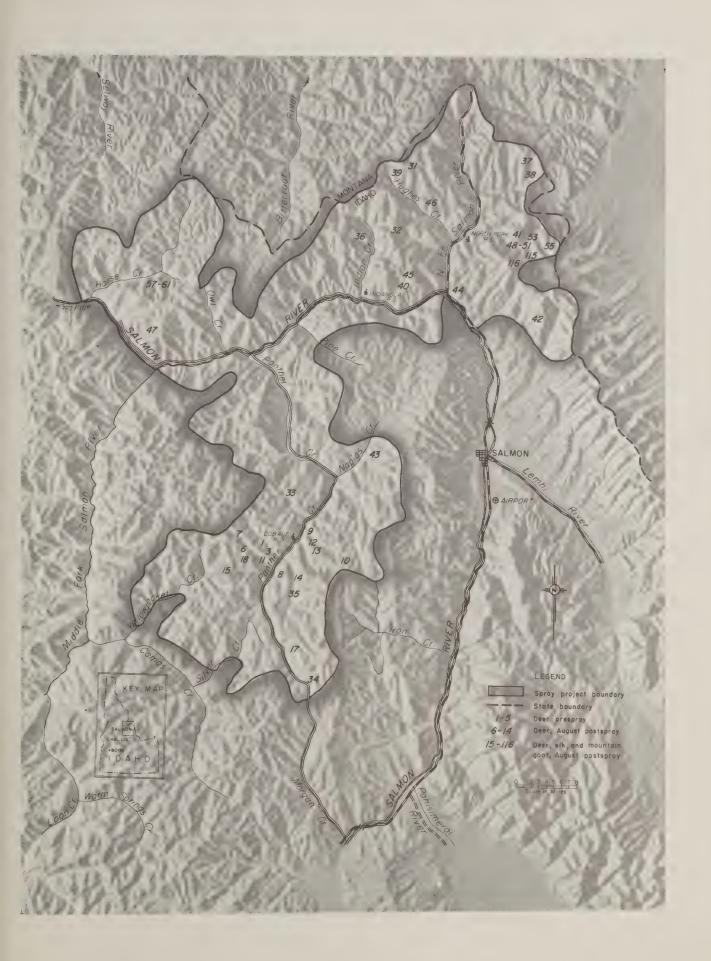
Extraction and Cleanup Procedure

Experimental work with some of the published procedures for the extraction of adipose tissues (beef fat), that had been fortified with known amounts of insecticides, indicated that the procedures were not adequate for this study. The following procedure was used after it was established that the adipose tissue samples could be essentially 100 percent extracted in this manner:

1. The samples minced with a sharp knife.

Figure 31. Numbers designate the collection sites of exposed big game animals.

¹ Agricultural Research Service mimeographed report PCY-64-17, 1964. (On file with Entomology Research Div., ARS, Yakima, Wash.)



- 2. Weigh out a 5-gram aliquot (take all of sample if original weight was less than 5 grams).
- 3. Blend for 2 minutes in a high speed Vitris blender using 100 ml. acetonitrile and 50 ml. n-hexane. After blending, 100 ml. acetonitrile was added.
- 4. Place blending jar and contents in 55° C. water bath for 2 hours. Stir continuously (n-hexane will be lost from sample during this time).
- 5. Filter through small cotton plug into separatory funnel. Rinse flask, plug, and funnel with 75 ml. n-hexane.
- 6. Shake sample with 200 ml. of distilled water and 50 ml. of saturated sodium sulfate.
- 7. Draw off water phase to second separatory funnel, wash with 50 ml. n-hexane and discard water phase.
- 8. Add 100 ml. of water to first separatory funnel, wash, transfer water phase to second funnel, wash, and discard water phase.
- 9. Combine n-hexane extracts and wash twice with 10 ml. of 2:1 concentrated; fuming sulfuric acid. Drain acid into second funnel, wash once with 50 ml. n-hexane and discard acid.
- 10. Combine n-hexane extracts and wash three times with distilled water, discard water phase and dry n-hexane phase with sodium sulfate.
- 11. Filter and evaporate to dryness and pick up in 1 ml. n-hexane.

Analytical Procedure

Gas chromatography using electron capture detection. (Research Specialties Model 600-B using Sr90).

Recovery

No tissue samples were avilable that were known to be insecticide-free. Where the original sample weight was large enough and analysis of a portion of the sample indicated a low level of residue, an additional aliquot was fortified with known amounts of each insecticide and the percentage recovery determined. When the sample was fortified at the 0.01 ppm level, the recovery was as follows: DDT-53%, TDE-103%, 0,p'-DDT 79% and p,p'-DDT 106%. The values reported have not been corrected for recovery values.

Comments

The percent of lipids (fats) extracted by the procedure used was determined for each sample and are reported. It is not intended that the percent of lipid reported represents all of the lipid present in the sample. It is intended that the percent of lipid reported represents a uniform portion of the total lipid content of the adipose tissue and as such can be used as a basis of comparison between samples.

An attempt was made to analyze a portion of the extract by a colorimetric method of analysis to confirm the values found by gas chromatography. Due to insufficient cleanup of the extract, the values found by colorimetric analysis were not reliable. Additional portions of adipose tissue have been obtained from samples 6, 7, 10, 12, and 14 and further attempts will be made to analyze these by means other than gas chromatography. The results of this portion of the study will be reported as an addendum to this report.

The presence of DDE and TDE, in the fat of man and animals, has been demonstrated by others. Barber and Morrison¹ recently demonstrated that TDE may possibly be a product of post mortem decomposition and may not occur in live tissues. The low levels of TDE reported may possibly be explained by the work of Barber and Morrison. The levels of DDE found in the post spray tissues may be explained by the fairly high levels of p,p'-DDT found in the tissue. The DDE that was found in the tissues was p,p'-DDE.

Detailed findings for the 14 animals are given in table 22.

¹Barker, P. S. and Morrison, F. O., 1964. *Breakdown of DDT to DDD in mouse tissue*. Can. J. Zoo. 42(2): 324-325.

Table 22. DDT and DDT metabolite residues in adipose tissues of prespray and 1-month postspray deer¹

Sample	Percent		Parts per mil	lion on adipos	e tissue basis		Total DDT
number²	extractable — lipids	DDE	TDE	o,p'-DDT	p,p'-DDT	Total	(100 per- cent lipids)
1	59.2	<.010	<.010	<.010	.012	.012	.020
2	44.2	<.010	<.010	<.010	.010	.010	.023
3	13.7	<.010	.010	<.010	.023	.033	.241
4	61.4	<.010	<.010	<.010	.010	.010	.016
5	52.4	<.010	<.010	<.010	.010	.010	.019
6	72.6	.36	.04	.70	9.78	10.88	14.91
7	63.8	1.70	<.010	2.97	38.42	43.09	67.22
8	63.4	1.48	<.010	2.98	16.18	20.64	32.40
9	84.2	.53	<.010	1.48	6.59	8.60	10.15
10	61.5	.85	<.010	4.04	14.00	18.89	30.60
11	54.0	2.12	<.010	2.34	44.68	48.14	90.91
12	75.2	1.27	<.010	2.34	17.87	21.48	28.35
13	53.5	.80	<.010	1.54	6.17	8.51	15.83
14	58.4	.10	<.010	.12	9.16	9.38	16.04

¹Agricultural Research Service mimeographed report PCY-64-17, 1964. (On file with Entomology Research Div., ARS, Yakima, Wash.)

An addendum was made to the above quoted report and read as follows:

As indicated in our Report no. PCY-64-17, additional analysis was to be done on adipose tissue samples to confirm the presence of DDT and DDT-metabolites.

Through the cooperation of the Fish and Game Department, State of Idaho, additional adipose tissues were obtained from five deer that were collected after the completion of the spray program.

Portions of these adipose tissues were analyzed by gas chromatographic techniques and by colorimetric procedures. After the samples had been extracted and cleaned up, to remove interfering materials, a small volume was analyzed by gas chromatography and the balance was analyzed colorimetrically. The following values were obtained:

Table 23. Check analyses results of some deer adipose tissues

Samula		illion of DDT metabolites	Percent
Sample - number	By gas chroma- tography	By colori- metric procedure	extractable lipids ¹
6	26.7	31.6	93.1
7	57.3	66.4	92.3
10	2.8	2	90.9
12	19.9	22.8	96.2
14	19.6	22.8	88.1

¹The extraction procedure was changed from that reported in PCY-64-17. In the above analysis the tissue sample was heated in chloroform before homogenizing thereby resulting in a higher efficiency of fat extraction.

²Numbers 1-5 were prespray. Numbers 6-14 were August postspray.

²The value found by the colorimetric procedure (0.4 ppm) was below the limit of accuracy of the analytical method as employed. The values obtained by the colorimetric procedure are 13 to 16 percent greater than the comparable gas chromatographic value. The values obtained by both procedures indicate that the adipose tissue samples contained high levels of DDT and its metabolites.

Adipose tissue samples numbered 15 through 99 were taken from animals harvested during the 1964 hunting season which extended from the last of August to the end of November. They were collected in the field by Idaho Fish and Game Department personnel and shipped to the Agriculture Research Service Laboratory. Handling procedures at the laboratory were slightly different than for the first 14 samples. The following procedures were used.¹

Extraction and Cleanup Procedures

Samples were removed from the freezer, cut into small cubes, and a representative portion removed for analysis. The sample was placed in a Vitris bowl, approximately 100 ml. CHCl₃ added, and the mixture heated to just below the boiling point of CHCl₃. The sample was then blended for 5 minutes, anhydrous sodium sulfate added, allowed to stand for approximately 5 minutes, and filtered through anhydrous sodium sulfate. Bowl and sulfate were rinsed with CHCl₃ to make a final volume of 200 ml.

A 100 ml. portion was washed 3X-10 ml. fuming (15%) sulfuric acid, 1X-20 ml. conc. sulfuric acid, washed with water until clear, a small amount of Celite 545 and sodium sulfate added and shaken for 1 min., and filtered through anhydrous sodium sulfate. The flask and sulfate were rinsed with CHCl₃. The filtrate was evaporated to dryness, rinsed with CHCl₃, evaporated, rinsed with n-hexane, evaporated, and made to 1 ml. with n-hexane.

Crude Lipid. A 5 ml. portion was removed from the 200 ml. volume and allowed to evaporate at room temperature in a tared beaker. The beaker was reweighed and the difference in weight used for determining the lipid content. Where there was an indication of CHCl₃ being trapped in the fat, the beakers were put in a 103° C. oven for 5 minutes, allowed to cool and the weight taken.

Analytical Procedure

Gas chromatography using electron capture detection. (Research Specialties Model 600-B using Sr 90). The following conditions were observed:

Column: 6 foot glass U-shaped,

packed with acid washed Chromosorb W, 3 foot coated with 5% DC-200 and 3 foot coated with 10% QF 1-0065.

210° C

Col. Temp: 210° C Det. Temp: 230° C

Gas Pressure: Nitrogen gas — inlet pres-

sure about 50 psi.

Recovery

Known amounts of DDE, TDE, o,p'-DDT and p,p'-DDT were added to samples to determine the efficiency of the extraction and cleanup methods. There were just a few control samples that had enough material to run a recovery and some of the spray samples that had enough material to run recovery and some of the spray samples that had enough material contained so much DDT that the amount added was not significant. The average recovery for DDT was 71.4% for deer, 127.5% for elk, 123.8% for goat. The average recovery for TDE + o,p'-DDT was 76.3% for deer, 97.5% for elk and 87.9% for goat. The average recovery for p,p'-DDT was 94.1% for deer, 128.2% for TDE + o,p'-DDT, and 101.7% for goat. TDE and o,p'-DDT could not be separated on this column.

A tabulation of DDT and DDT metabolites in the 1964 hunting season big-game adipose tissues is presented in table 24.

The average and range in residue levels of adipose tissue for all big game animals sampled are shown in table 25.

During the hunting season of 1962, personnel of the Idaho Fish and Game Department collected adipose tissue samples from 98 deer, 43 elk, and 1 mountain goat at various points throughout Idaho. Tissues were sent to the Agricultural Research Service

¹Agricultural Research Service mimeographed report PCY-65-16, 1965. (On file with Entomology Research Div., ARS, Yakima, Wash.)

Table 24. DDT and DDT metabolite residues in adipose tissues of big game taken during the 1964 hunting season¹

Sample	Lipid content		Residues ² (part	s per million)		Total DDT
number	(percent)	DDE	TDE+o,p'-DDT ³	p,p'-DDT	Total DDT	- (100 percent lipids)
Deer from	project area:					
15	81.7	.66	3.33	13.05	17.04	20.85
16	89.7	2.61	7.86	118.18	128.65	143.42
17	87.2	.03	.11	.46	.60	.69
18	95.1	.35	1.22	25.58	27.15	28.55
32	89.3	2.00	.60	15.40	18.00	20.16
37	77.6	.17	1.98	7.84	9.99	12.87
38	87.3	.09	.77	3.51	4.37	5.00
39	82.3	.29	1.00	8.60	9.89	12.02
40	90.0	.60	2.42	20.20	23.22	25.80
41	75.4	.10	.22	3.44	3.76	4.98
42	73.3	.40	1.60	16.20	18.20	24.83
43	92.1	.25	.90	5.05	6.20	6.73
44	80.4	.05	.22	2.96	3.23	4.02
45	89.4	.76	3.44	31.28	35.48	39.69
46	81.5	.02	.05	.67	.74	.90
51	74.0	<.01	.24	3.04	3.29	4.46
Elk from p	project area:					
31	75.1	16.00	2.36	66.00	84.36	112.32
33	80.3	.07	.38	3.65	4.10	5.11
34	76.8	<.01	.63	1.10	1.74	2.25
35	90.8	1.00	1.98	14.10	17.08	18.83
36	92.8	2.44	2.42	26.80	31.66	34.13
47	91.6	3.62	4.22	35.84	43.68	47.69
48	80.3	<.01	.04	.24	.29	.35
49	73.6	.03	.12	1.22	1.37	1.86
50	78.3	.02	.04	.56	.62	.80
53	72.8	<.01	.09	.43	.53	.71
55	99.9	.15	.78	9.20	10.13	10.14
115	93.2	1.20	6.58	18.80	26.58	28.52
116	80.0	<.01	3.60	12.20	15.81	19.75
	goat from project	area:				
57	89.2	3.64	7.26	28.40	39.30	44.06
58	83.2	4.60	11.30	44.80	60.70	72.96
59	81.4	1.88	8.49	33.96	44.33	54.46
60	72.1	3.10	6.80	24.01	33.91	47.03
61	82.2	4.88	9.68	33.24	47.80	57.76
	control areas:					
71	91.0	<.01	<.01	<.01	<.01	<.01
72	74.4	<.01	<.01	.01	.01	.01
74	96.0	.09	<.01	.08	.17	.17
75	97.4	.03	<.01	.06	.09	.09

Table 24. (continued)

Sample	Lipid content		Residues ² (part	s per million)		Total DDT
number	(percent)	DDE	TDE+o,p'-DDT3	p,p'-DDT	Total DDT	- (100 percent lipids)
76	92.4	.04	<.01	.06	.10	.11
77	90.2	.01	<.01	.04	.05	.05
78	94.0	.01	<.01	.03	.04	.04
79	94.7	.01	<.01	.02	.03	.03
80	86.0	.03	<.01	.02	.05	.06
81	89.7	.03	<.01	.04	.07	.08
82	84.5	.04	<.01	.04	.08	.10
83	91.1	.05	<.01	.03	.08	.09
84	94.9	.06	<.01	.07	.13	.13
85	86.5	.06	.02	.11	.19	.22
86	90.5	.03	<.01	<.01	.03	.03
87	86.2	.10	<.01	.04	.14	.17
88	94.6	.01	<.01	.01	.02	.02
89	96.4	.02	<.01	.06	.08	.08
90	90.2	.02	<.01	.05	.07	.08
92	97.7	.02	<.01	.05	.07	.07
93	97.2	<.01	.01	.02	.03	.03
94	95.3	.01	<.01	.01	.02	.02
95	93.3	.01	<.01	.01	.02	.02
96	90.2	.02	<.01	.03	.05	.05
97	91.6	<.01	<.01	<.01	<.01	<.01
98	74.4	<.01	<.01	.25	.25	.34
99	75.1	.01	<.01	.20	.21	.28
Elk from o	control area:					
70	72.1	<.01	<.01	.03	.03	.04
73	73.5	<.01	<.01	.03	.03	.04
Mountain	goat from contro	l areas:				
19	80.7	<.01	<.01	.05	.05	.06
20	72.0	.01	.01	.03	.05	.06
22	81.0	.01	.01	.07	.09	.11
23	79.6	.01	<.01	.04	.05	.06
24	87.5	.01	<.01	<.01	.01	.01
25	87.0	<.01	<.01	.01	.01	.01
26	70.6	<.01	<.01	.01	.01	.01
27	75.9	<.01	<.01	<.01	<.01	<.01
28	71.8	<.01	<.01	.01	.01	.01
29	84.7	<.01	<.01	.02	.02	.02

 $^{^1}Agricultural\ Research\ Service\ mimeographed\ report\ PCY-65-16,\ 1965.$ (On file with Entomology Research Div., ARS, Yakima, Wash.)

 $^{^2}Based$ on adipose tissue samples as received and not corrected for the average recovery. The minimum sensitivity of the method as used is 0.01 ppm for all compounds.

³TDE+o,p'DDT could not be separated by the analytical method employed and are calculated as one.

Table 25. Average and range in residue levels of adipose tissue from all big-game animals sampled

posts			Kesidu	Residues ¹ in parts per million	million		Total DDT
August postspray from 9 Deer 9 Deer 27 Deer 27 Deer	percent	DDE	TDE	o,p'-DDT	p,p'-DDT	Average Total DDT	(100 percent lipids)
August postspray from 9 Deer 1964 hunting season f	rea						
August postspray from 9 Deer 1964 hunting season f	46.2 13.7 - 61.4	<.01	.002	 	.013	.015	.063
9 Deer 1964 hunting season factor for the season factor factor for the season factor f	spray area						
1964 hunting season fi 27 Deer 2 Elk	65.2 53.5 - 84.2	1.02	.004	2.06	18.10 6.17 - 44.68	21.18	34.04
	rom control area	318					
			23				
	90.2 74.4 - 97.7	.03	<.01		.05	.08	.011
	72.1	<.01	<.01		.03	~	
Goat	79.1 70.6 - 87.5	<.01<.0101	<.01 <.01 <.01		.03	.03	.03
1964 hunting season from spray area	rom spray area						
16 Deer	84.1 73.3 - 95.1	.52	1.62		17.22	19.36	22.16
13 EIK	83.5 72.8 - 99.9	1.88	1.79		14.63	18.30	21.72
5 Goat	81.6 72.1 - 89.2	3.62	8.70 6.80 - 11.30		32.88 24.01 - 44.80	45.20 33.91 - 60.70	55.26 44.06 - 72.96

¹These have not been corrected for the average recovery. ²TDE and o,p'-DDT could not be separated by the method used, and are calculated as one.

Laboratory where they were analyzed for DDT and DDT metabolites. No samples were taken from animals known to have been directly exposed to an insecticidal spray program.

That same year, personnel of the Washington State Department of Game collected adipose tissue samples from 102 deer, 82 elk, and 9 mountain goats. These also were taken from areas where there were no recent direct exposures. The tissue samples also were sent to Agricultural Research Service Laboratory for analyses of DDT and DDT metabolites.

Residue levels found in both the Idaho and Washington samplings may be considered as norms, or controls. This gave an additional basis for comparison with controls taken in conjunction with this 1964 spray project. Data gathered from the Idaho and Washington samplings are summarized in table 26. (Note that results in this table are based on 100 percent extractable lipids.)

The first portion of the table indicates specific animals taken during 1962 from the 1964 spray project area.

As a basis for comparison, residue levels

Table 26. DDT residue levels in non-exposed big-game animals taken in Idaho and Washington in 1962

Deer sample	Sex	Estimo	ated	Percent			llion based e xtractable li	
number		Weight	Age	lipids	DDE	TDE	DDT	Total
Nule deer sar	mpled in	1962 from th	ne 1964 Sc	ılmon spray a	rea¹			
67	М	_	1 1/2	47.3	Т	Т	.08	.08
68	М	140	2 1/2	56.9	T	Т	.04	.04
69	М	145	2 1/2	32.6	.06	Т	.21	.27
71	М	140	2 1/2	46.9	Т	Т	.08	.08
72	М	140	2 1/2	64.4	Т	Т	.10	.10
73	М		2 1/2	56.1	.02	Т	.07	.09
Average:					.013	Т	.097	.11
Spec	ies	No. of samples						
Statewide av	erages, la	daho, 1962¹						
Deer		98		47.2	.0004	.020	.223	.2434
Elk		43		53.2	.007	.074	.145	.226
Mountai	n goat	1		60.4	Т	Т	.080	.080
Speci	es	No. of samples						
statewide av	erages, V	Vashington,	1962²					
Deer		102		50.7	.004	.020	.266	.290
Elk		82		43.3	.005	.084	.160	.249
Mountai	n goat	9		37.9			.070	.070

T = Any value less than .01 part per million.

¹Agricultural Research Service mimeographed report PCY-63-13, 1963. (On file with Entomology Re-

search Div., ARS, Yakima, Wash.)

²Agricultural Research Service mimeographed report PCY-63-12, 1963. (On file with Entomology Research Div., ARS, Yakima, Wash.)

found in big-game animals taken during monitoring this and other aerial DDT spray projects have been summarized in table 27. Included in the tabulation are summaries of the 1962 Idaho and Washington statewide collections referred to previously, but values are on adipose tissue basis, not corrected for extractable lipids.

It is apparent from data presented in the foregoing tables that most wild game animals, regardless of where taken, have measurable amounts of DDT residues present in their adipose tissue. Amounts seldom exceed 1 ppm in areas where animals have not been directly exposed to a DDT spray program, at least in recent years.

As a result of spraying in the 1964 Salmon project, there were mule deer, elk, and mountain goats that had higher residue levels than from any other known project area reported in the literature. The average for deer taken during the hunting season was slightly lower than the average of those taken 2 months earlier. The highest level (128.65 ppm) was, however, almost three times greater than the highest August sample (48.14 ppm).

Idaho Fish and Game Department personnel said that elk taken late in the hunting season from the North Fork area had probably migrated from Montana into the Salmon National Forest during the fall. If so, these animals naturally received less exposure to DDT spray. Before analyses were made, the probable migratory elk (numbers 48, 49, 50, 53, 55, 115, and 116) were designated by biggame biologist Ralph Pehrson. Grouping the spray area elk accordingly, the average results are shown in table 28.

Nonmigratory elk reflect the expected additional exposure, averaging about four times as high as the migratory elk. This average, 30.44 ppm, is higher than for deer taken either in August or during the hunting season.

Residue levels in mountain goats far exceeded earlier conjectures, especially since the average was higher than for either deer or elk. Local grazing and feeding habits could account for the difference. All mountain goats sampled from the project area were shot near one hunting camp in the Horse Creek area. In the Horse

Creek drainage the terrain is quite rugged and interspersed with spruce budworm infested timber types, good semi-open game feeding areas, and rocky outcroppings. Because of the infested timber, the entire area, except stream protection zones, was sprayed. The area, although below the subalpine zone, is excellent mountain goat habitat and the animals make use of it even during the summer season. All animals would thus have received about equal exposure.

The spray project evidently caused the DDT residue level in adipose tissues of many exposed game animals to rise sharply above normal. No acute toxicity cases were detected.

No information was gathered in this monitoring program to evaluate or predict the subtle long-range physiological reactions of animals acquiring the increased residue levels.

Residues in Other Tissues of Big Game Rumen Content

Rumen content samples were obtained from four deer taken prior to spraying and from five animals collected 1 month after spraying (samples numbered 2-10, table 29). Free liquids were squeezed from all samples. Samples were then frozen and transported to the Denver Laboratory of the Bureau of Sport Fisheries and Wildlife. There they were thawed, air-dried, and ground up in a blender. Proportionate parts from the five prespray samples were mixed to make one composite sample. Rumen contents from the five postspray deer were analyzed as individual samples.

Extraction and analysis procedures and results were reported to the Forest Service.¹

Residue extractions were made by using a solvent of 5 percent methanol in iso-octane. Extractions were than cleaned with Florisil. They were analyzed by gas chromatography

¹Correspondence from Glen Crabtree to Floyd Iverson, January 7, 1965.

Table 27. DDT residue levels in adipose tissues of big-game animals taken in 1964 compared with findings in other studies from sprayed and unsprayed areas

Reference	cg.	ιδ	10	ນ	ນາ	4 P	4C
DDT applied (Ibs./acre)	.75	1.0	1.0	1.0	1.0 .		
Size Spray project (M-acres)	12	74	120	430	88		
Range of levels (ppm)	.26-10.48 .09-10.18 .037190 .180260	0.0-9.0	15.0-27.0	0-21.6	0-42.2	<.0134 <.01-3.04 <.0158 <.0109	<.0123 <.0107 <.01-1.92 <.0233
Average DDT ¹	2.73 2.99 .097 .22	9.0 6.0 5.	0 21.5 .5	6.0	12.7	.5 .134 .095	.103 .042 .121 .106 .05
Number of animals	12 ² 25 ³ 8	7	044	24	10	13 102 82 9	4 4 8 8 F L E
Months after spray	4 4 4 4	5 13 17	3-4	1-5	4 4		
Pre or post spray	post post post	post post post	pre post	post	post		pre pre pre pre pre pre pre pre pre pre
Species	Deer Elk Deer Elk	Deer Deer Deer	Deer Deer Deer	Elk Deer	Deer Elk	Bear Deer Elk Mt. goat	Antelope Bear Deer Elk Mf. goat Moose
Year	1963 1964 1964	1959 1960 1960	1960 1960 1961	1962	1962	1962	1962
Area	Washington (Hemlock- looper)	Montana (Bitterroot)	Montana (Gallatin)	New Mexico (Santa Fe)	Colorado (Rio Grande)	Washington (statewide)	ldaho (statewide)

Table 27. (continued)

Area	Year	Species	Pre ar posí spray	Months after spray	Number of animals	Average DDT ¹	Range of levels (ppm)	Size Spray project (M-acres)	DDT applied (lbs./acre)	Reference
Idaho (Salmon)	1964 1965 1966 1964	Deer Deer Deer Deer Elk	pre post post post post	1 3 3 3	5 9 13	.013 21.07 19.36 18.30	.01033 8.51-48.14 .60-128.65	540	1.0	79
l Idaho (Salmon controls)	1964	Mt. goat	post	7	٧٠	45.20	33.91-60.70			4
Pahsimeroi Boise & Elmore Boise	1964	Mt. goat Deer Elk			10 28 2	.03 .03	<.0109			4

¹Average total DDT residues in parts per million, not corrected for average recovery nor to 100 percent extractable lipids.

²Eight of these deer were taken within the sprayed area or within I mile of the sprayed area.

Seventeen of these elk were taken within the sprayed area or within 1 mile of the sprayed area.

⁴The following are Agricultural Research Service mimeographed reports on file with Entomology Research Div., ARS, Yakima, Wash. aReport PCY-64-8, 1964.

bReport PCY-63-12, 1963.

cReport PCY-63-13, 1963.

dReport PCY-64-17, 1964.

From Pillmore and Finley, 1963. eReport PCY-65-15, 1965.

Table 28. Average and range of DDT residues in migratory and nonmigratory elk from the spray area

Type of elk	Number of samples	Lipid content (percent)	Total DDT (tissue basis, parts per million)	Total DDT (100 percent lipids, ppm)
Migratory	7	82.6	7.90	8.88
		72.8-99.9	.29-26.58	.35-28.52
Nonmigratory	6	84.6	30.44	36.72
		75.1-92.8	1.74-84.36	2.25-112.32

Table 29. DDT residues in deer rumen content samples

Commiss	Dana assault an	Residues (parts per million)							
Samples	Deer number	p,p'-DDT	DDE	o,p'-DDT	TDE	Total ND 155.6 185.3 27.0 75.8			
Prespray	2, 3, 4 & 5	ND1	ND	ND	ND	ND			
Postspray	6	125.0	5.6	16.0	9.0	155.6			
n'	7	150.0	6.3	19.0	10.0	185.3			
"	8	16.0	Tr. ²	2.4	8.6	27.0			
"	9	53.0	3.2	7.6	12.0	75.8			
"	10	130.0	3.1	4.0	15.2	152.3			

 $^{{}^{1}\}underline{N}D = \underline{Not} \ detected.$

with the Dow 11 column and a QF-1 column with an electron capture detector.

Residue amounts were calculated on the basis of known amounts of reference standards interspersed in the sample series. Prespray samples were run at the same sensitivity as were the other samples.

A comparison is made in table 30 of the DDT residue levels found in the adipose tissues and rumen content samples from nine deer. No direct correlation can be drawn. Information does indicate that 1 month after spraying, the deer had accumulated high levels of DDT residues in their fat tissues and exposure was still high as evidenced by the residue levels in the rumen samples.

Thyroid, Adrenal, Liver, and Kidney

Samples of liver, kidney, thyroid, and adrenal tissues were taken from eight deer (samples numbered 2-10, table 21). Tissues

Table 30. Comparison of DDT residue levels in adipose tissues and in rumen contents of deer

Door seemals	Total DDT in po	arts per million
Deer sample number	Adipose tissues ¹	Rumen content ²
Prespray:		
2, 3, 4 & 5		ND ³
2	.010	
3	.033	
4	.010	
5	.010	
Postspray (1	month):	
6	10.88	155.6
7	43.09	185.3
8	20.64	27.0
9	8.60	<i>75</i> .8
10	18.89	152.3

¹From table 22.

 $^{{}^{2}}Tr. = Trace = \langle 1.0 \ ppm.$

²From table 29.

 $^{^3}ND$ = No detection. This represents a composite of four samples.

were frozen and transported to the Bureau of Sport Fisheries and Wildlife, Denver Laboratory.

Analyses of these tissues will be made for residues of DDT and DDT-metabolites. This work was not completed at the date of this report. Results from only four animals were available before going to press (table 31).

BIRDS

Time and personnel limitations precluded the possibility of making an intensive ecological evaluation of the spray project impacts on birds, their productivity, or population structure. After consultation with several professional people on this phase of evaluation, is was decided to limit the work on birds to studies on robins (*Turdus migratorius*) and grouse, and taking a general census.

Robins

This species was chosen as representative of songbirds which might be affected by DDT spray. Five birds were collected on June 25 before the spray project started (fig. 33). It was planned that at each 15-day period after spraying up to five birds would be taken. Time permitted taking only a total of seven birds for postspray samples.

The gizzard of each bird was taken for analysis as a separate sample. Birds utilizing DDT killed insects would be expected to have higher concentrations of residues in their gizzards than in their carcasses. Therefore, inclusion in total body concentrations could distort the total residue values because of some of the DDT in gizzard materials would have been excreted and not absorbed. The rest of the body, except for beak, feet, skin, and feathers, was used as the carcass sample. Each sample was placed in a plastic bag, labeled, frozen, and shipped to the Agricultural Research Service Laboratory for analysis. Extraction, cleanup, analytical, and recovery procedures used were as follows:1

Figure 32. Fourteen of the mule deer taken were to sample adipose tissue, adrenal and thyroid glands, liver, kidney, and rumen content. The big-game biologist and the conservation officer of the IF&G Department and the research biologist of the BSF&W provided assistance in this sampling.



Extraction and Cleanup

Samples were removed from the freezer and a portion was placed in a Virtis bowl, approximately 100 ml. chloroform added and the mixture was heated to just below the boiling point of chloroform. The sample was then blended for 5 minutes, anhydrous sodium sulfate added, allowed to stand for approximately 5 minutes and then filtered through anhydrous sodium sulfate. The bowl and sulfate were rinsed with chloroform to make a final volume of 200 ml. A one hundred milliliter portion of the chloroform extract was evaporated to dryness with air in a warm water bath. The residue was taken up in 20 mls. of distilled acetonitrile,

¹ Agricultural Research Service mimeographed report PCY-65-20, 1965. (On file with Entomology Research Div., ARS, Yakima, Wash.)

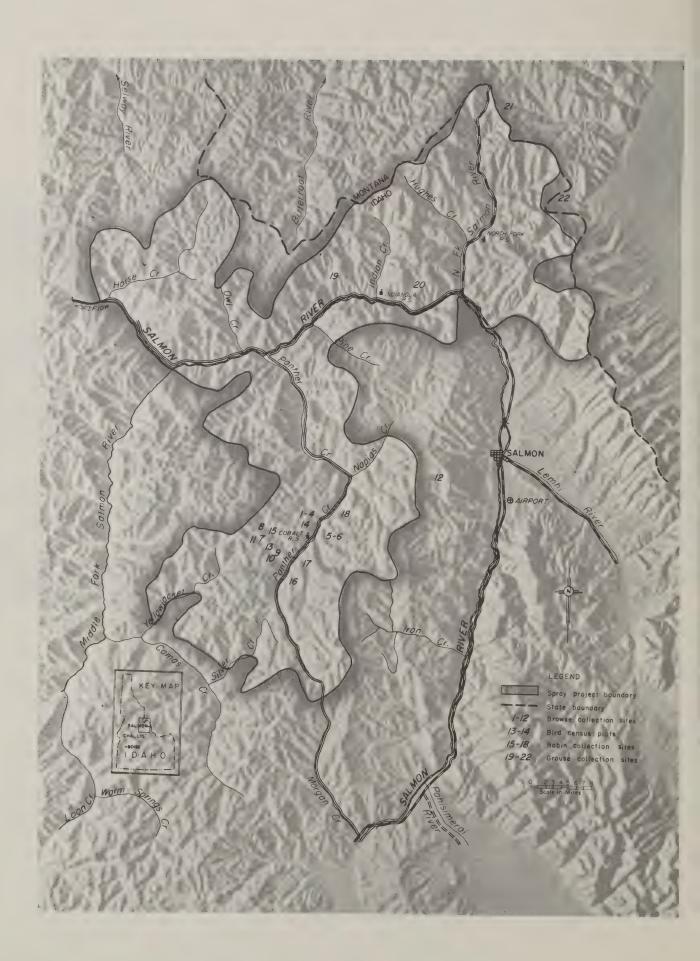


Figure 33. The numbers indicate robin, grouse, and vegetation collection sites and bird census sites.

heated on a steam bath and anhydrous sodium sulfate added and then cooled. It was then filtered through a cotton plug overlayed with anhydrous sodium sulfate into a chromatographing column of 15 grams of a 15-1 mixture of Florisil and Nuchar C-190-N. The original flask was washed with 20 mls. acetonitrile and the funnel with an additional 10 mls. acetonitrile. When the acetronitrile level reached the top of the column (Top just barely dry), 50 mls. of a mixture of $1 \div 1$ distilled n-hexane and chloroform were added. To the eluant was then added 35 mls. water, 10 mls. saturated sodium sulfate solution, and 40 mls. distilled n-hexane and the solution was shaken for one minute. When the layers had separated, the lower layer was aspirated off and the remaining hexane layer was washed with two additional washes of water and then dried with anhydrous sodium sulfate and filtered. The filtrate was then evaporated to dryness with air in a water bath and taken up in a measured volume of distilled n-hexane.

A 5 ml. portion of the chloroform extract was removed from the 200 ml. volume and allowed to evaporate to dryness at room temperature in a weighed beaker. The beaker was then reweighed and the difference in weight was the crude lipid content of the sample. Where there was an indication of cholroform being trapped in the fat, the beaker was put in an 103° C oven for five minutes, allowed to cool, and weighed again.

Analytical

Gas chromatography using electron capture detection. Research Specialties Model 602 B-Sr 90 detector.

Column: 6-foot glass U-shaped, packed with acid washed Chromosorb W, and of which 3 feet are coated with 5% DC-200 and 3 feet coated with 10% QF 1-0065. Column Temperature: 210° C. Sr 90 Detector Temperature: 230° C. Gas Pressure: Nitrogen gas—inlet pressure about 50 psi.

Recoveries

It was difficult to determine the recovery accurately on robins both because of lack of sample and because some of the samples that had to be used contained too much insecticide to measure small amounts accurately. For some as yet unexplained reason, the acid method used on the big game animals gave zero recoveries of DDE. The method finally used gave better recoveries but was still not as good as desired. By the method finally used, the recoveries averaged 36% for the DDE and 54% for the combined TDE and o,p'-DDT and 91.8% for the p,p'-DDT. There was not enough material to run recoveries on the gizzards.

Results of the analyses are shown in table 32.

Grouse

Bird biologists of the Idaho Fish and Game Department were interested in a limited study on probable effects of this spray project on blue grouse (*Dendragapus obscurus*) productivity.

Collection of up to five spray area female blue grouse and the same number from a control area outside the project boundaries during the fall of 1964 was planned. Approximately the same number from the same areas were planned for at prenesting or nesting time in the spring of 1965. It was also planned to collect clutches of blue grouse eggs from the spray area and from a control area to be hatched and observed.

Because of a general decline in blue grouse populations in the project area the past few years, only three birds were obtained during the fall of 1964 (fig. 33), and no spring collections were made in 1965. The three bird carcasses were sent to the Bureau of Sport Fisheries and Wildlife in Denver. Each carcass, minus feet, beak, skin, and feathers, was ground up, blended, frozen, and shipped to

Table 31. Residues (parts per million) of DDT and DDT metabolites in thyroid, adrenal, liver and kidney tissues of deer¹

Deer sample number ²	p,p'-DDT	o,p'-DDT	TDE	DDE	DDD	Total DDT
Thyroid:						
6	Т					Т
7	.59	3.00				3.59
8	.60	.60				1.20
9		1.39				1.39
Adrenals:						
6		8.10				8.10
7	Т	Т	1.30			1.30
8	.60	3.00				3.60
Liver:						
6				3.85		3.85
7			1.92			1.92
8					1.92	1.92
Kidney:						
6	Т		Т	Т		Т
7	.48					.48
8	Т					Т
Visceral fat:						
6	16.00					16.00
7	48.00					48.00
Subcutaneous	fat:					
6	16.00					16.00
7	36.40					36.40
Unclassified fa	ıt:					
8	12.00					12.00

SUMMARY

	Thyroid	Adrenals	Liver	Kidney	Visceral fat	Subcutaneous fat	Unclassified fat
6	Т	8.10	3.85	Т	16.00	16.00	
7	3.59	1.30	1.92	.48	48.00	36.40	
8	1.20	3.60	1.92	T			12.00
9	1.39						

¹Results reported by correspondence from Crabtree, BSF&W, to Casebeer. All results determined by paper chromatography on basis of frozen tissue weight after thawing.

²Results from only four animals available before going to press.

the Agricultural Research Service Laboratory. Extraction, cleanup, and recovery procedures were the same as used for robins. The acid method also could not be used on the grouse. The recoveries by the method used were 132 percent for DDE, 108.5 percent for the com-

bined TDE and o,p'-DDT, and 136 percent for p,p'-DDT. Table 33 lists results of DDT residues found.

During the regular forest grouse hunting season in Montana, personnel of the Montana Fish and Game Department killed four blue

Table 32. Residues of DDT and DDT metabolites in robins¹

		-	Crude		Residues (parts per million) ²				
Sample number		Type of sample	lipid (percent)	DDE	TDE+ o,p'-DDT	p,p'-DDT	Total DDT	and range total DDT in carcass	
#1 - Prespray		Carcass	6.0	.64	.17	<.10	.81		
		Gizzard	3.4	.42	<.10	<.10	.42		
#2 - Prespray		Carcass	10.4	.26	<.10	<.10	.26		
		Gizzard	6.6	.11	<.10	<.10	.11		
#3 - Prespray		Carcass ³	6.2				_ (1.165	
		Gizzard	7.6	.19	.14	.12	.45	.26-3.13	
#4 - Prespray		Carcass	5.2	3.13	<.10	<.10	3.13		
		Gizzard	4.5	2.92	<.10	.10	3.02		
#5 - Prespray		Carcass	4.3	.46	<.10	<.10	.46		
,		Gizzard	4.5	<.10	<.10	<.10	<.10		
							1		
	#1	Carcass	4.9	1.50	.98	.88	3.36		
First Postspray,		Gizzard	4.1	.62	.62	.54	1.78		
July 24	#2	Carcass	6.9	2.60	.37	.70	3.67		
		Gizzard	9.0	1.22	.33	.80	2.35		
	Ш. •		2.7	1.83	.71	.24	2.78		
Second Postspray,	#1	Carcass Gizzard	12.3	.63	.39	.52	.77	3.16	
August 19								3.10	
Trelore Gulch	#2	Carcass	3.4	2.63	.68 .97	.55 2.00	3.86	.62-5.85	
		Gizzard	1.9	1.50	.97	2.00	4.47	.02 0.00	
	#1	Carcass	5.0	4.94	.39	.53	5.85		
	., -	Gizzard	1.9	.72	.10	.27	1.09		
Third Postspray, September 3 Panther Creek Drainage	#2	Carcass	4.1	.32	.12	.18	.62		
		Gizzard	7.2	.18	<.10	.10	.28		
	#3	Carcass	7.2	1.17	.23	.57	1.97		
	71 0	Gizzard	9.0	.73	.18	.39	1.30		
							1		

¹Agricultural Research Service mimeographed report PCY-65-20, 1965. (On file with Entomology Research Div., ARS, Yakima, Wash.)

²Results not corrected for average recovery. Minimum sensitivity for detection of the method used was 0.1 ppm.

³Sample lost when first method of cleanup did not work.

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Table 33. Residues of DDT and DDT metabolites in three postspray blue grouse¹

		Residues (parts per million)						
Sample	Crude lipid (percent)	DDT	TDE and o,p'-DDT	p,p'-DDT	Total DDT	Corrected total DDT (100% lipids) ²		
A Sage Creek August 19	1.3	5.83	1.30	.71	7.84	602.8		
B Spring Creek August 30	1.0	6.87	.63	.86	8.36	836.0		
C Spring Creek November 20	11.7	5.63	2.83	4.08	12.54	107.1		
Average:					9.58			

¹Agricultural Research Service mimeographed report PCY-65-20, 1965. (On file with Entomology Research Div., ARS, Yakima, Wash.)

Table 34. Residues of DDT and DDT metabolites in forest grouse collected as part of a Montana study¹

Grouse species			Collection date ² (1964)	Crude - lipid (percent)	Residues (parts per million)					
	Sex	Age			DDT	DDE	DDD	Total DDT	Corrected total DDT (100% lipids) ²	
Blue	М	juv.	9-13	64.0	72.0	64.0	6.9	142.9	222.9	
Blue	М	2+	9-19	79.8	61.0	156.0	8.0	225.0	281.3	
Blue	F	juv.	9-23	80.9	46.0	116.0	2.7	164.7	204.2	
Blue	М	juv.	9-23	70.1	22.2	27.8	.9	50.0	72.8	
Franklin	М	2+	9-23	76.5	6.2	3.8	0.6	10.6	13.9	
Franklin	М	2+	9-23	57.9	11.3	15.7	1.0	28.0	48.4	
Franklin	M	2+	9-19	78.5	42.0	105.0	15.0	162.0	205.7	
Franklin	F	1+	9-23	69.3	117.0	166.0	15.0	298.0	429.1	
Franklin	F	juv.	9-19	86.0	40.0	40.0	14.0	94.0	109.0	
Franklin	М	juv.	9-19	82.6	7.0	4.4	1.5	12.9	15.6	
Ruffed	F	juv.	9-23	78.3	45.0	81.0	4.0	130.0	166.4	

¹Unpublished data reported in correspondence from Tom Mussehl to Robert Casebeer, 5-3-65.

²Corrected to 100 percent lipid basis by the author.

 $^{^2}Birds$ taken 9-13 and 9-19 were from site No. 21 and those taken 9-23 were from site No. 22, indicated in figure 33.

³Corrected to 100 percent lipid basis by the author.

grouse, six Franklin grouse (Canachites franklini) and one ruffed grouse (Bonasa umbellus). They were all taken on the Idaho-Montana state line on or near the edge of this spray project boundary (fig. 33, numbers 21 and 22). Fat tissue was taken from each of these birds and sent to the Wisconsin Alumni Research Foundation Laboratories, Madison, Wisconsin. They were analyzed by gas chromatography for residues of DDT and DDT metabolites. Results are shown in table 34. The figures in the corrected total DDT column were computed and added in this report.

Bird Census

Before the spray project started, it was the consensus of most workers in the area that the population of insectivorous songbirds in the Salmon National Forest was higher than normal. If such were the case, the adnormal food supply, spruce budworm, could have accounted for the increased number of birds. One species, the western tanager (Piranga ludoviciana), was quite prominent in numbers. Increased numbers of this species were also noted elsewhere in the Rocky Mountain region during the same spring and summer seasons. This phenomenon is known to occur occasionally since this species seems subject to population fluctuations. The same phenomenon may hold true for other less conspicuous species.

As indicated previously, no elaborate ecological appraisal was made of bird populations in relation to the budworm infestation or to the spray treatment. A technique was planned and tested on the project, however, to give a possible indication of acute impacts.

Two contrasting timber types were selected for study. One was an open-canopy Douglas-fir stand with a southwest exposure on Porphyry Ridge (No. 13, fig. 33). The other was a closed-canopy lodgepole pine (*Pinus contorta*) -alpine fir (*Abies lasiocarpa*) stand on an easterly exposure of Blackbird Ridge (No. 14, fig. 33). In each area, a 40-acre square plot was delineated by string-lining the perimeter. Five counting-route center

lines were flagged or string-lined across each plot, thus providing an observation strip 2 chains wide on each side of the center line.

Birds were counted by traversing each 4chain wide strip in a serpentine manner, recording those birds observed within the strip.

Counts were made in early morning, usually starting about 6:00 a.m. This took advantage of the birds most active period and gave optimum light for identification. Only clear, calm mornings were selected for counting.

Prespray counts were made 6 and 7 days before, and postspray counts were made 3 and 4 days after the respective areas were treated. The counts are shown in table 35.

Twenty-one bird species were identified on these plots. The Oregon junco, (Junco sp.) chipping sparrow (Spizella passerina arizonae), western tanager, (Piranga ludoviciana), and Cassin's finch (Carpodacus cassini) composed the majority of observations. Approximately a dozen other species were identified within the spray project area but outside the census plots. Possibly some of them were represented as "unidentified" within the plots.

The counts represent samples of bird occurrence in the plots. They show that no drastic reductions in bird numbers occurred during the census interval, thereby indicating no acute population change as a result of spraying. Actually, there was a slight increase in numbers. One reason could have been the increased activity of fledglings and females occurring at this particular time. Singing activity, noted by the observers, was greater during censusing following treatment than previous to spraying.

Careful observation over routes traveled failed to reveal dead birds within the plots. None were reported over the remainder of the project area although intensive searches were not conducted.

VEGETATION

Although the project operational plan designated open meadows of more than 160 acres as nonspray types, much forage grows among semi-open timber types and was thus

¹Correspondence from Tom Mussehl, Montana Fish and Game Department to the author, 5-3-65.

Table 35. Prespray and postspray bird counts on two 40-acre plots

	Plot	Porphyry Rie	dge (No. 13)	Blackbird Rid	lge (No. 14)
	Туре	Prespray	Postspray	Prespray	Postspray
	Date (1964)	<i>7</i> -11	7-21	7-12	7-22
Name of birds	Time (a.m.)	6:00-7:55	5:55-7:50	5:45-7:30	6:00-7:45
Jnidentified		17	27	9	18
Junco sp.		3	2		3
Robin Turdus migrate		1			
Pine siskin Spinus pinus p		1			
Mountain blue bird Sialia currucoid		2			
Canada jay Perisoreus cana		1			
Chipping sparrow Spizella passeri	na arizonae	21	29	5	4
Cassin's finch Carpodacus cas					1
Western tanager Piranga ludovid		2	5	1	2
Mountain chickade Penthestes gan		1			1
Red breasted nuth Sita canadensis			1		
Hairy woodpecker Dryobates villo		1			
Blue grouse Dendragapus o		3			
ranklin's grouse Canachites fran			3		
Hermit thrush Hylocichla gutt	ata				1
vening grosbeak Hesperiphona v				5	2
Downy woodpeck Dryobates pube		2			
Audubon warbler Dendroica audu			2	1	1
Red shafted flicke Colaptes cafer	r	3			1
ong sparrow Melospiza melod	dia	2			
Williamson's sapsu Sphyrapicus th	yroideus			2	1
led-tailed hawk Buteo borealis				1	
Totals:		60	69	24	35

exposed to insecticide spray. Thus, livestock and wildlife feeding on this vegetation were exposed to the DDT. Vegetation was therefore sampled to determine the persistence of DDT residues to species of forage. The study was not designed to inventory or measure distribution patterns of insecticide on vegetation.

Prespray vegetation samples were collected between July 1 and 6 at 21 sites within the upper Panther Creek drainage. Control samples were collected in the Williams Creek drainage. Ten species were sampled from the Panther Creek sites and nine of those same species were sampled from the control site. Each species from each collection site was held separately.

Species sampled were:

Big sagebrush — Artemesia tridentata
Douglas-fir — Pseudotsuga menziessi
Snowberry — Symphoricarpos spp.
Balsamroot — Balsamorhiza sagittata
Snowbrush — Ceanothus velutinus
Current — Ribes sp.
Chokecherry — Prunus demissa
Bluebunch wheatgrass — Agropyron inerme

Idaho fescue — Festuca idahoensis

Bitterbrush — Purshia tridentata

Just before spray application, five oil-sensitive dye-cards were placed approximately 1 chain apart on a transect through each collection site within the project area. After spraying, the cards were evaluated for the amount of spray reaching ground level. Cards from 10 sites indicated that no spray, or only trace amounts, had reached the ground. All prespray collections from these sites were discarded, and these 10 sites were eliminated from consideration for postspray collecting. Average of dve-card readings from the other 11 sites showed from .06 to 1.24 pounds of DDT per acre had reached the ground. Thus, these were selected as the treatment sites (table 36 and fig. 33).

Repeat collections were made from the same project and control sites of the same species at four postspray periods:

1. July 19 and 21, immediately following spray application.

- 2. August 18 and 19, about 1 month after spraying.
- 3. September 22.
- 4. October 27.

Not all species were collected from each site. Table 36 describes the location of each site and indicates species sampled.

Current annual growth was selected for all collections. Each sample of each species was put in an individual, open-mesh, regular 10-pound potato bag, hung in a building, and allowed to air-dry for 1 to 2 weeks. Each sample was then ground in a blender. Control collections were maintained individually by species for each collection period, totaling nine prespray and 36 postspray samples. Equal amounts of foliage were taken from each project sample. Composite samples were made of each species for each collection period, totaling 10 prespray and 40 postspray samples for the project area.

All collections were shipped to the Bureau of Sport Fisheries and Wildlife Laboratory in Denver for analyses.

Residue analyses of four species were made by chemists of the Denver Laboratory. Extractions and analyses were made as follows:¹

After air-drying, grinding, and compositing the necessary samples, they were prepared and analyzed in the following way:

- 1. Residue extractions were made of each sample by use of a solvent of five percent methanol in iso-octane.
- 2. Extractions were cleaned with Florisil.
- 3. Analysis made by gas chromatography with Dow 11 column and electron capture detector.
- 4. Amounts were calculated on the basis of known amounts of reference standards interspersed in the sample series. Prespray samples and control samples from unsprayed areas were run at the same sensitivity as were the samples from the sprayed areas.
- 5. Analyses were made for p,p'-DDT, for DDE, and for the combined o,p'-DDT and TDE.

¹Correspondence from Glen Crabtree to Floyd Iverson, January 7, 1965.

Table 36. Vegetation sampling sites and species

						Speci	es co	llecte	d			
Site Numbe	er Location	Avg. dye-card reading ¹	Big sagebrush	Douglas-fir	Snowberry	Balsamroot	Bitterbrush	Snowbrush	Currant	Chokecherry	Bearded blue- bunch wheatgrass	Idaho fescue
1	Dummy Creek — 1.9 miles from Blackbird road junction where road passes over ridge and turns right. Site is on south slope.	1.24	Х	Х	Х	Х	Х				Х	
2	Dummy Creek — .3 miles up road from No. 1. Site is on right.	.60	Х	X	Х	Х	Х				Χ	
3	Dummy Creek — .3 miles up road from No. 2. Site is on right.	.36		Х	Х	Х				Х	Χ	
4	Dummy Creek — .8 miles up road from No. 3. Site is on right.	.83						X				
5	Copper Creek — 1 mile up South Fork Fawn Creek road from its junc- tion with Copper Creek. Site is on right.	.09	Х	Х	X	Х	Х				X	X
6	Copper Creek — .9 miles up road from No. 5. Site is on right.	.27	Χ	Χ	Х	X	X				X	X
7	Musgrove Creek — First canyon on right up drainage from Withington Cabin. Site is on left .5 miles up road from junction.	.30	Х	Х	X	Х	Х				X	X
8	Musgrove Creek—200 to 300 yards up road from a point .2 miles beyond No. 7. Site is on left.	.07;	X	X	X	Х	Х				X	
9	Porphyry Ridge — In saddle on ridge between Musgrove and Porphyry Creeks at upper end of meadow. 1.8 miles up road from Forney.	.81	X	X fe	X	Х	Х				X	X
10	Porphyry Ridge — .3 miles beyond No. 9 on lower road. Site is on right near last salt block.	.09	Χ	Χ		Х	X				X	X
11	Porphyry Creek — 5 miles up can- yon from Panther Creek junction. Site is on steep south slope on right.	.06	X	X	X	X			X		X	
13	Control, Williams Creek, directly east of Forest Service cabin.		X	X	X	X	X	X		X	X	Х

 $^{^1}Equals$ pounds of DDT to the ground per acre as determined from average of readings of five dye-cards per site.

Figure 34. Total DDT residues are summarized for four species of vegetation (for 1964).

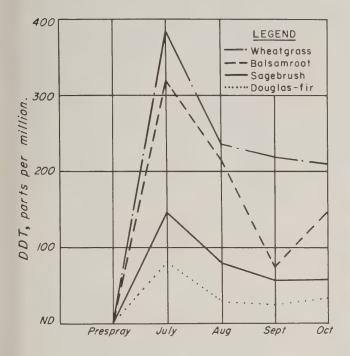


Figure 35. Current annual growth from samples of 10 vegetation species was clipped, airdried in mesh bags, ground up in a blender, put in plastic bags, and sent to the BSF&W laboratory to analyze for DDT residues.



Findings from analyses of the four species which have been completed were transmitted in the same correspondence and are shown in table 37. Analyses for August 1965 samples for two species also are shown in the same tabulation.

A summary of total DDT for the four species is illustrated by figure 34. Results from the other six species will be reported when available.





Table 37. DDT residues (parts per million) in four species of vegetation¹

Sample	Year month	p,p'-DDT	DDE	o,p'-DDT & TDE	DDMU	Total
		Big s	agebrush	ø,		
	(1964)					
Prespray	June-July	ND^2	ND	ND		ND
Postspray	July	128.0	4.2	16.0		148.2
Postspray	August	69.0	3.1	8.0		80.1
Postspray	September	53.0	1.3	3.5		57.8
Postspray	October -	55.0	1.6	2.5		59.1
Control	July-October ³	ND	ND	ND		ND
	(1965)					
Postspray	August	.1	.1	.1	2.0	2.3
Control	August		Tr. ⁴	Tr.	.2	.2
		Bals	amroot			
	(1964)					
Prespray	June-July	ND	ND	ND		ND
Postspray	July	279.0	5.9	28.0		312.9
Postspray	August	196.0	2.1	17.0		215.1
Postspray	September	64.0	2.7	9.7		76.4
Postspray	October	127.0	3.3	19.0		149.3
Control	July-October ³	ND	ND	ND		ND
		Bluebuncl	n wheatgras	s		
	(1964)					
Prespray	June-July	ND	ND	ND		ND
Postspray	July	317.0	7.1	60.0		384.1
Postspray	August	202.0	3.5	33.0		238.5
Postspray	September	186.0	2.8	32.0		220.8
Postspray	October	170.0	3.2	38.0		211.2
Control	July-October ³	ND	ND	ND		ND
		Dou	glas-fir			
	(1964)					
Prespray	June-July	ND	ND	ND		ND
Postspray	July	64.0	2.3	13.0		79.3
Postspray	August	30.0	ND	ND		30.0
Postspray	September	27.0	ND	ND		27.0
Postspray	October	30.0	Tr.	5.3		35.3
Control	July-October ³	ND	ND	ND		ND
	(1965)					
Postspray	August	19.0	Tr.	Tr.		19.0
Control	August	ND	ND	ND		ND

 $^{^1}Correspondence$, Glen Crabtree to Floyd Iverson and Robert Casebeer. $^2ND=Not$ detected. 3Composite samples of five collection periods. $^4Tr=Trace$, <1.0 ppm.

Variation of levels between species at the first postspray sampling has no obvious cause with which it can be associated. Major reduction in levels, between 29 and 62 percent, occurred within the first month after spraying. Reductions in levels, from 43 to 76 percent, depending on the species, continued during the second month. In only one species, wheatgrass, was there a further decrease the third month, and that was very slight. Increased levels occurred in balsamroot, sagebrush, and Douglas-fir, but they all ended the 3-month postspray period with residue levels

about half that detected immediately following spraying.

Differences in the rates of changes in residue levels between species could most likely be associated with moisture and tissue differences caused by different rates and degrees of curing through the summer and fall. This could result in concentration variations with the presence of the same total residue. There is also the possibility of variations in spray concentration on different portions of sampled plants and the resulting sampling of different applied rates.

AGRICULTURAL PRODUCTS

Various state and federal agencies have legal responsibilities for checking contaminates in agricultural products. These duties are assigned either by state or federal laws. As monitoring programs by other agencies were already in effect, arrangements were made with key individuals of those agencies to extend their programs into the project area, or to intensify programs already in effect there. They were asked to evaluate, as completely as possible, the impact of this particular spray program on those environmental components under their jurisdiction.

All agency representatives contacted were very cooperative in providing assistance. Sampling programs were established for the various products. Some laboratory analyses were financed by the Forest Service; while others were supplied free.

The project area is not an intensive agricultural area, there being a limited number of producers with few products. Producers reside mostly along the bottom lands of major streams and rivers. Within the project area normal protection was afforded most private lands either because they were within nontype areas for spraying or by being inside the protective nonspray widths stipulated along water courses. Special protection was provided by designating private lands as nonspray zones. In situations where infested timber types occurred on private lands Forest Rangers contacted the landowners. Infested timber on private lands would then be designated

for spray or nonspray, according to the landowner's wishes. Similar to stream protection measures, 400-foot wide strips adjacent to nonspray areas were sprayed with helicopters at the rate of 0.5 pound DDT per acre.

Products of concern were cream marketed at the Salmon creamery; Grade A milk sold to a processing plant in Missoula, Montana; beef cattle grazed on National Forest allotments; and hay produced on a farm near the Salmon airport.

CREAM

The State Department of Agriculture has responsibilities in Idaho for administering standards of marketing cream. Contacts were made with the Director of Dairying and with the Regional Dairy Inspector and a program developed for sampling cream produced within the project area and in outlying areas beyond exposure.

The three general sampling areas were the North Fork of Salmon River, Lemhi River valley between the towns of Salmon and Lemhi, and the main Salmon River above Salmon along the Salmon-Challis highway. Only the North Fork was within project boundaries.

Three individual producers were chosen from each area. The Dairy Inspector took 2 ounces of cream, as samples, from each producer's supply three different times. The samples from the three producers in each area were combined to make a composite sample

Table 38. Results of composite cream sample analyses

Area of collection	Pres _i		Fii posts samp	pray	Seco posts samp	pray
	Date	DDT ¹	Date	DDT ¹	Date	DDT
No. Fork of Salmon	6-9-64	Neg.	7-29-64	Neg.	11-16-64	Neg.
Lemhi Valley	11	Neg.	"	Neg.	"	Neg.
Salmon to Challis	11	Neg.	"	Neg.	ıı .	Neg.

¹DDT and DDT metabolites results less than 1 part per billion are reported as "Neg." (negative)

totaling 6 ounces for each of the three collection periods. Results can only represent a composite of producers.

One prespray and two postspray collections, 1 and 5 months later, were made by the Dairy Inspector for Lemhi County (table 38). Samples were sent to Boise where they were analyzed by the Idaho Department of Agriculture chemist. Results reported as negative indicate less than 1 part per billion DDT.

GRADE A MILK

The State Department of Health has responsibilities within Idaho for administering Grade A milk standards. The Salmon area is not a dairying area. Only a few producers supply milk for regular bulk tank pickup. The milk is taken to a processing plant in Missoula, Montana. There were no Grade A milk producers within the project area, but there were three north of Salmon, from 1 to 6 miles outside the project boundary, and two others south of the town, where there was no exposure.

The Chief, Laboratories Section, and the District Supervising Sanitarian of the Idaho Department of Health, were contacted and a program for sampling Grade A milk from each of the five producers was developed. Samples were taken before spraying, about 3 weeks after spraying, and about 2 months after spraying. The District Sanitarian made the

collections and shipped the samples to the state office in Boise. There they were analyzed in the same laboratory shared with the State Department of Agriculture. All services of the Department of Health for collecting samples and laboratory analyses were contributed. A listing of collections and results of analyses appears in table 39.

BEEF

There are 21 cattle allotments on the three Ranger Districts of the Salmon National Forest within the project boundaries. Some 30 permittees graze nearly 2,200 head of cattle on these allotments starting as early as May 1 and extending as late as November 15. During the winter previous to spraying, the forest supervisor, range staff officer, and respective district rangers met with those livestock associations having permittees on those allotments and explained the spray project proposal and various implications involved. There was no voiced objection to the project and the permittees were not particularly concerned about ultimate impacts under normal grazing procedures.

Although many animals were subject to exposure, certain built-in protection factors tended to dilute exposure intensity. Other factors inherent to the cattle producing industry in the Salmon area provided additional dilution by marketing time. These protection factors were:

Table 39. Results of Grade A milk sample analyses

Area of collection	Pres samp	pray bling	post	rst spray pling	posts	ond spray oling
•	Date	DDT ¹	Date	DDT ¹	Date	DDT ¹
Adjacent to project:						
10 mi. N. of Salmon	6-16-64	Neg.	7-29-64	Neg.	10-13-64	Neg.
7 mi. N. of Salmon	"	Neg.	"	Neg.	"	Neg.
4 mi. N. of Salmon	"	Neg.	"	Neg.	"	Neg.
Distant from project:						
1 mi. S. of Salmon	"	Neg.	"	Neg.	"	Neg.
3 ½ mi. S. of Salmon	"	Neg.	"	Neg.	"	Neg.

¹Results of less than 1 part per billion shown is "Neg." (negative)

- 1. Nonspray types of more than 160 acres were not treated. Large range types, thus exempted, constituted the more intensively used portions of allotments.
- 2. Bottom lands along streams with a flow of more than 5 cfs were protected with 400 feet on either side as nonspray and an additional 400 feet of only 0.5 pound DDT per acre. More than 700 miles of stream bottoms were designated for this protection. Stream bottoms constitute a major portion of cattle range within forested types.
- 3. Portions of most allotments were outside the project area.
- 4. At the time of spraying (July) vegetational development at higher elevations of the project area was not complete. DDT is not known to be systemic and would not translocate to portions of the plant which grew after spray application. Cattle are normally moved to higher elevations after vegetation has made additional growth. This was done after spraying was completed.
- 5. Relatively few cattle remain on forest allotments late in the season. Some are removed as early as July 31, others by the end of August, and the majority are gone by mid-September. Thus, for most cattle, 2 to $2\frac{1}{2}$ months was the maximum time of exposure.
- 6. Ordinarily, cattle from forest allotments are taken to farmlots for supplemental feeding before marketing. Thus, additional time was provided, whereby losses of at least a portion of accumulated residues could occur.

These dilution factors cannot be taken for granted as resolving the problem of DDT exposure, however.

Originally, plans for evaluating spray impact on beef cattle called for a periodical biopsy of cattle exposed in the project area under normal grazing conditions. It seemed to be too sensitive an area, public relations wise, to expect permittees to subject their stock to biopsy procedures. It would have been best to have had cattle available solely for this type of an evaluation. Such stock would not

be subject to marketing or other outside controls. This alternative became evident too late to explore the legal procedures which would allow the Forest Service or another agency to acquire livestock for this purpose.

An acceptable substitute plan was devised The Meat Inspection Division of the Agricultural Research Service has the responsibility of inspecting meat and meat products processed and sold by the City Packing Company in Salmon. A full-time ARS veterinarian is the Federal Meat Inspector. He agreed to obtain adipose tissue samples from local cattle if processed in this packing plant.

There was no schedule whereby beef producers in the Salmon area could be expected to market beef animals through this packing plant. Occasionally culls or cripples could have been processed during the summer. Additional animals could be expected at the end of the grazing season or after fall or winter feeding.

Two lists of beef producers' names were furnished the Federal Meat Inspector. One list was of permittees having cattle on allotments within the project area. The other list was of Salmon National Forest permittees whose allotments were not exposed to spraying. If cattle from any of these producers were slaughtered at this plant, the Inspector was to take adipose tissue samples and send them to the ARS Laboratory in Yakima. For various reasons, no cattle from the listed producers were processed through the plant.

A sample of only one beef animal was obtained elsewhere. This was taken on February 4, 1965, approximately $4\frac{1}{2}$ months after the animal was taken off National Forest range. Analysis findings were as follows:

Percent crude lipid was 78.4 percent. DDE in parts per million was 2.35 as received and 3.00 on a 100 percent lipid basis. TDE + o,p'-DDT in parts per million was 2.25 as received and 2.87 on a 100 percent lipid basis; p,p'-DDT in parts per million was 0.59 as received and 0.75 on a 100 percent basis. Total DDT in parts per million was 5.19 as received and 6.62 on a 100 percent lipid basis.

HAY

The primary agricultural pursuit in the Salmon area is ranching. Within the project area a few ranchers raise hay only for their own stock needs. Protection measures applied to these areas have been described previously. No comments were received from ranchers within the project area during or after the project to indicate concern about hay contamination.

One situation developed adjacent to the airport. A small rancher immediately south of the airport was cutting, baling, and stacking alfalfa hay during spraying operations. The contractor who furnished the spray mixed the materials on the south edge of the Salmon airport grounds. After mixing a large volume of spray concentrate, he had an accumulation of DDT container bags which he burned near

his installation. The Salmon Forest Supervisor received reports from the rancher's wife that ashes from the fire had blown onto the hay field. She was concerned about contamination of the hay as they planned to sell it. By this time the hay had been baled and stacked. Approximately 9 tons of hay were involved.

A representative of the Idaho State Department of Agriculture was contacted and on September 14, he visited the ranch. Two samples of the hay were made from composite cores taken from 10 bales, representing a total of approximately 400 bales. The two samples were sent to Boise and analyzed for chlorinated hydrocarbon residues by the State Department of Agriculture chemist. Results were reported to the Forest Service as negative. This information was passed on to the rancher. He expressed satisfaction with the test results.

SPECIAL SITUATIONS

CULINARY WATER

Specifications of the aerial spray contract provided for special protection measures for streams and lakes. In applying these protective measures particular attention was given to culinary water supplies, especially those drawn from open streams.

Domestic water for the town of Salmon is taken from Jesse Creek, a drainage flowing towards Salmon from the west. Most of the drainage area is inside the Salmon National Forest, but none was within the spray project area. Jesse Creek was in the logical flight line between much of the project area and the Salmon airport. It was thus vulnerable to accidental spray deposition if there were no restrictive controls. The entire drainage of Jesse Creek (fig. 36) was, therefore, designated as a nonflight zone. This area was drawn on all project maps and in briefing and orientation flights for pilots and observers, special emphasis was devoted to its protection. Return flight patterns for planes coming from north and northwest of Salmon were restricted to flying across the lower end of Jesse Creek below the point where the town's water supply enters an underground system.

Observers and other project personnel were careful to watch the flight patterns. No violations of the Jesse Creek restrictions were observed.

Numerous ranches and summer homes in the general area are supplied with water from open streams. Such streams were normally included within zones provided for aquatic environment protection. Some water ditches, especially along the North Fork and lower tributaries of the Salmon River, meandered outside the normal protection zone. In such cases the protective zones were extended to include the ditches.

The communities of Gibbonsville and Cobalt obtain water from reservoirs supplied by open streams within the project area. Gibbonsville water is drawn from the Anderson Creek reservoir (fig. 36) which is supplemented at times from Dahlonega Creek by a ditch. Nonspray zones were extended to provide protection for both the Anderson Creek reservoir and the ditch from Dahlonega Creek. Regular stream protective measures were applied to the upper portions of these drainages.

The Cobalt water supply is taken from the Spring Creek reservoir (fig. 36). The non-spray zone was widened to include the reservoir area and normal protective measures were applied to the remainder of Spring Creek above the reservoir.

A small store at Forney (fig. 36), at the junction of Porphyry Creek with Panther Creek, is supplied with water directly from Porphyry Creek. Regular protective measures were applied to the entire length of Porphyry Creek and its main tributaries.

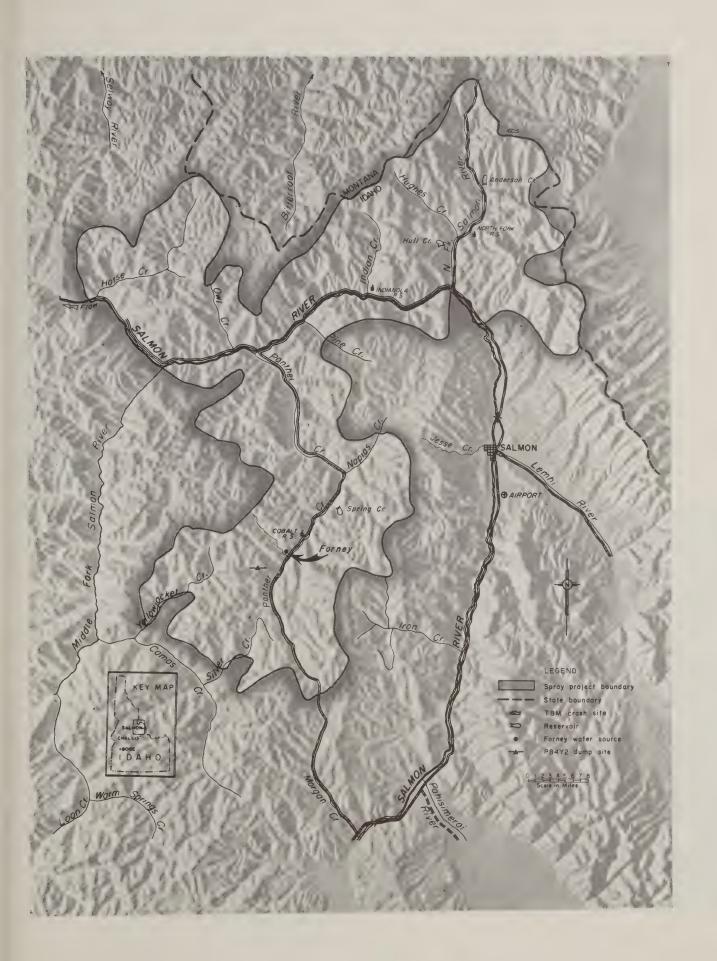
The Idaho State Department of Health administers prescribed purity standards of water for public use throughout Idaho. Arrangements were made whereby their personnel, with help of Forest Service personnel, would take prespray and postspray samples of water from the three supplies mentioned. The samples were sent to Boise and analyzed for DDT residues by the Department's chemist. Analysis results are listed in table 40.

HULL CREEK RESERVOIR

Hull Creek is a tributary of the North Fork of Salmon River. At the junction of the South Fork of Hull Creek with the main creek a reservoir of about 10 surface acres in size has been constructed (figs. 36 and 37), mostly on private land. A resort has been established there which caters primarily to people who fish this reservoir.

Terrain-wise, the reservoir is situated at the bottom of a basin-like merging of drainages. Normal air drainage in such a situation

Figure 36. The locations of special situations occurring during the project are shown.



would carry volatilized spray materials from upper elevations to the bottom of the basin and deposit them on the reservoir. Waterflow from the reservoir is small and unsteady (because of a regulating weir) so a water turnover is not assured. Under these conditions, spray materials getting into the water would be cumulative. Experience elsewhere had indicated that pesticides accumulated in impounded water have caused severe delayed effects on aquatic life.

This particularly sensitive situation demanded special protective measures for this drainage. A 1000-foot fringe around the reservoir was designated as a nonspray area as were both sides of the main creek for some distance above the reservoir. Most of the remaining drainage was sprayed by helicopter with 0.5 pound DDT per acre. This action assured that the planes would not have to fly or turn over the reservoir or the major length of the stream.

Water samples were taken at three locations early in the morning of July 8, before helicopter spray operations started. These locations were: (1) main Hull Creek, just before the stream entered the reservoir; (2) surface of the reservoir approximately midway along the north side; and (3) where water from a spring in the South Fork of Hull Creek entered the reservoir.

Samples were taken again at about 3:00 p.m. the same day. Residue analyses were made and reported by the Agricultural Re-

Figure 37. Hull Creek Reservoir is situated in a basin-like location. It is a private resort depending on the reservoir fisheries resources. This made extra precautions imperative. Nonspray and helicopter widths were widened as added precautions against possible accumulation of spray in impoundment.



search Service. All results were less than 0.2 parts per billion, or negative within the limitation of analyses.

No reports have been received to date of any adverse effects or reactions. Evidently the special protective measures were adequate.

Table 40. Results of culinary water sample analyses

Area of collection		pray	posts	rst spray ection	posts	ond pray ection
	Date (1964)	DDT ¹ Residue	Date (1964)	DDT ¹ Residue	Date (1964)	DDT Residue
Spring Cr. reservoir (Cobalt)			7-20	Neg.	9-15	Neg.
Anderson Creek reservoir (Gibbonsville)	7-10	Neg.	7-21	Neg.	9-13	Neg.
Forney store	6-30	Neg.			9-15	Neg.

¹Results of less than 1 part per billion shown is "Neg." (negative)

EMERGENCY SPRAY RELEASES

Specifications for the aerial spray contract in the project operational plan included the following statement:

In case emergency dumping of insecticide becomes necessary, dumping will be done away from waterways, residences, and pastures, if at all possible.

Project personnel recognized the possibility that emergency situations might make it necessary to dump spray materials. No advance planning can specifically outline monitoring for such emergency situations. Techniques and methods are tailored to specific incidents as they occur.

Early on the first day of helicopter operation (July 2), one spray pilot, operating from the helispot at Shoup along the main Salmon River, jettisoned part of his load. The Monitor Coordinator made the investigation that afternoon. The pilot had found himself in a tight spot without sufficient room to maneuver safely. He felt it was not safe to try flying out without dumping the remaining 30 gallons of spray. He jettisoned from a low elevation near the top of the ridge almost directly across the Salmon River from Shoup. There is no running water at the site, the nearest being the Salmon River, one-fourth mile away. It was felt that this concentrated spray dose would have no particular impact on the forest environment.

The danger in such a situation is in the public relations aspect. If an incident is not properly investigated and reported immediately, rumors of damage start to circulate and the situation is soon magnified out of all proportion to the facts.

Other incidents occurred the same day pertaining to helicopter operations. One involved the helicopter pilot spraying along Horse Creek. He had trouble with the motor furnishing the spray boom pressure. There were no emergency helispots available along Horse Creek; so he landed on a gravel bar where Horse Creek joins the Salmon River. In trying to correct the trouble some spray mixture

was pumped through the booms and released directly onto the sandbar. Rotor-blade action also blew some finer spray particles out over the Salmon River.

Repetitions of this situation occurred at two other helispots along the Salmon River. The river canyon in places is very narrow and often the only helispots available were on the bank immediately adjacent to the river (fig. 38). On a number of occasions, the pilots primed the spray apparatus by hovering low over the helispots, with the rotor blades fanning finer spray out over the Salmon River.

These incidents were brought to the attention of helicopter pilots, observers, foremen, and contractors during the briefing session held later that day. Strict instructions were

Figure 38. The helispot at Bear Gulch is on the main Salmon River. The terrain is rugged and access by servicing vehicles very restricted. It was necessary to work very close to the river and fly under hazardous topographic conditions.



issued that system priming was not to be done on helispots close to open water or domestic developments. The pilots were instructed to move their craft to spray areas for priming.

Two spray planes crashed during the project. Each case was investigated to determine the insecticide load disposition and the possible impacts it might have on various resources.

PB4Y2 Crash

On July 17, the four-engine PB4Y2 made an emergency crash landing at about 6:40 a.m. on an open hillside outside the project area. First reports indicated the pilot had jettisoned a partial load somewhere in the upper reaches of the Panther Creek drainage. At about 10:00 a.m., the Project Leader dispatched the Forest Service Fishery Biologist to monitor Panther Creek for possible impacts. A monitoring station was established about 3 miles above the mouth of Panther Creek and approximately 32 miles from the suspected jettison area. Waterflow was computed at 382 cubic feet per second at that station. Water samples and aquatic insect drift were taken at 1/2 hour intervals from 12:30 p.m. through 6:30 p.m. Additional individual water samples were taken during the afternoon by the fishery biologist at other points in Panther Creek.

Samples were taken at 5:20 p.m. above the mouth of Musgrove Creek, 5:40 p.m. above the mouth of Blackbird Creek, and 6:00 p.m. above the mouth of Napias Creek.

Upon return of the Monitor Coordinator from the field at noon, he was notified of the crash and was assigned to investigate the jettisoned load. The fishery and big-game biologists of the Idaho Fish and Game Department were notified and briefed.

Computations by the observer assigned to the PB4Y2 indicated that between 350 and 730 gallons of insecticide had been dumped.

The spray plane pilot, the observer, and the observer plane pilot each reported different locations for the jettisoned load. An intensive search in these areas was made.

At 1:00 p.m. on July 18, the site was found about ½ mile up Fourth of July Creek (fig. 36). This is a small drainage which empties into Panther Creek from the west about 1 mile above Forney and about 35 miles above the mouth of Panther Creek. Spray materials were spread over an area approximately 200 feet long and 150 feet wide. Fourth of July Creek was running a maximum flow of 5.2 cubic feet per second that day, and ran almost through the center of the dump site. There was no question that insecticide had entered the stream, but it was impossible to determine how much.

Additional water samples were taken at 2:05 p.m. on Fourth of July Creek about ¼ mile below the site, and 2:40 p.m. on Panther Creek about ½ mile below Fourth of July Creek.

No water for any type of domestic use was being taken from Fourth of July Creek. Cattle were not seen near the drop site when it was found. The forest ranger indicated the stock was supposed to have been moved to another unit of the allotment several days before. Later reports indicated that cattle were seen in the immediate area, however.

Idaho Fish and Game Department personnel were informed the next morning of finding the site. The two biologists were taken for an aerial flight over the area to note the exact site. Plans were then formulated for additional monitoring of the effects on the aquatic environment.

On July 20, Forest Service crews used an electric shocker to sample wild fish from just below and just above the drop site on Fourth of July Creek, and from Panther Creek, about ½ mile below and ½ mile above the mouth of Fourth of July Creek. Aquatic submergent vegetation samples were taken at each of the same four locations. In addition, aquatic insect bottom samples were taken from 10 square feet of stream bottom in each of the four locations. The same type of sampling was done at the same locations during October 19-22, 1964.

Water, fish, and aquatic vegetation samples were sent to the Agricultural Research Service Laboratory for analysis of DDT residues.

Results of all sampling are as follows:

- 1. Lower Panther Creek monitoring station. Thirteen water samples representing each ½ hour period from 12:30 p.m. to 6:30 p.m. on July 17, showed less than 0.2 ppb DDT per sample. Aquatic insect drift samples showed no increase in numbers over this same period of time. This monitoring station was about 32 miles below the spray dumpsite. It is probable that water passing the site when the dump occurred (approximately 6:35 a.m.) had not reached the monitoring station by the end of the collecting period. Spray materials either had not reached the station or had been so diluted that impacts could not be detected.
- 2. Water samples. Other samples taken on

- July 17 and 18 showed less than 0.2 ppb.
- 3. Wild fish, aquatic vegetation, and bottom aquatic insects sampling. Results are reported in table 41.

There is little question but what a substantial amount of DDT entered Fourth of July Creek and caused higher contamination of aquatic life than found anywhere else within the project. Both wild fish and aquatic vegetation in the stream immediately below the dump site had a high level of residue contents when sampled 3 days after the jettisoning. Levels continued to rise sharply during the next 3 months.

This same pattern did not occur in Panther Creek just below Fourth of July Creek. Although levels started out at about 2 ppm for fish and aquatic vegetation just after jettison, there was an insignificant rise for fish and 50 percent reduction for aquatic vegetation in the following 3 months. Dilution by a much larger volume of water running

Table 41. DDT residues in wild fish and aquatic vegetation, and the number of bottom aquatic insects sampled in conjunction with jettisoned load of insecticide on Fourth of July Creek¹

			Fish		Aquatic	Bottom
Location	Date (1964)	Fish sample description	Crude lipids (percent)	Total DDT (ppm)		aquatic insects Total numbers
Fourth of July Creek						
Above dump	7-20	Rainbow	4.0	2.02	.212	108
	10-19	3 rainbow, 1 cutthroat, 3 Dolly varden	3.3	3.22	.291	374
Below dump	7-20	Rainbow	2.9	10.96	3.65	104
Dorom domp	10-19	3 rainbow, 1 cutthroat	3.7	26.24	11.04	111
Panther Creek Above 4th of July						
Creek	7-20	Rainbow	3.0	1.82	.256	30
	10-19	4 rainbow	2.4	.446	.086	33
Below 4th of July						
Creek	7-20	Rainbow	4.0	2.28	1.76	26
	7-20	Whitefish	6.7	2.64		
	10-19	2 whitefish, 1 rainbow	2.1	2.56	.085	

¹Agricultural Research Service mimeographed report PCY-65-14, 1965. (On file with Entomology Research Div., ARS, Yakima, Wash.)

in Panther Creek no doubt reduced the impacts of the high concentration of DDT which came out of Fourth of July Creek.

Aquatic insects did not react as might be expected. The increase above the dump site could have been expected. A lack of any significant change below the dump site cannot be explained, especially since numbers remained at three times those found any time on either Panther Creek site. Panther Creek exhibited small populations both times in both locations. This creek is known to be quite sterile, however.

TBM Crash

On July 20, 1964, one of the TBM planes landed about 100 yards north of the upper end of a tributary drainage on the north side of Dahlonega Creek (figs. 36 and 39). The crash site was in Montana just outside the project area.

The pilot had made only one short spray run after reloading, so his plane contained almost a capacity load of 700 gallons when it crashed.

Figure 39. The TBM crash site, looking from Montana side south towards the tributary of Dahlonega Creek where pilot had been spraying. Helispot cleared for access to crash site.



The first investigator at the crash site reported that the pilot was killed and the entire spray load had been carried with the plane into the crash site. There was no open water close by, and no spray could possibly have reached open water. This was verified by the Monitor Coordinator and Forest Service Fishery Biologist during a flight over the area.

TEST USE OF BURETTES FOR WATER SAMPLING

Several methods are used to take samples of running water for analyses of contaminaants. In standing water the problem of sampling is easily resolved since stable contaminants will usually remain in place for long periods of time. In running water, however, the problem of proper sampling becomes complicated because foreign materials are usually flushed rapidly downstream. In addition, they may have entered the water at several points along a stream causing the materials to drift downstream in separate bodies or blocks. Each block may vary in concentration, and within each block a gradation in concentration may be expected. The size or length of blocks can also vary.

If the sampling procedure consists of dipping a water sample from a stream at determined intervals of time, several difficulties arise. A dip sample taken at regular intervals may miss a moving block of material or may be taken when a diluted portion of the concentration is moving through the sampling station. In streams with exceedingly fast flow velocities, the first possibility becomes a high probability unless the sampling frequency is increased. If the numbers of samples are increased, laboratory analyses costs will also increase, however.

The use of devices such as burettes or charcoal filters could solve some of the problems of water sampling for insecticide materials. Charcoal filters placed in a stream could yield a continuous sample. But, procedures for extracting DDT from the charcoal are complicated and the rate of recovery is not consistent. Use of a burette is advantageous in that it may be calibrated to take desired

volumes of water over predetermined time intervals. A trial was made during this project to compare continuous burette stream sampling with dip sampling.

Burettes used for this testing were cylindrical pyrex funnels with open top and stopcocks. To make sure of continuous water mixing in the top of the burette, a large top with a 125 milliliter capacity was used. The burette was attached to a board for the purpose of anchoring. A rubber tube was attached to the bottom of the funnel to direct the water into a collection container. The device was then anchored at a point in the stream allowing enough drop in elevation for gravity flow from the burette to the collection container. The stop-cocks were so graduated to yield samples of 1 pint of water over a 15-minute period, or 1 gallon in one hour.

Burette samples collected July 9 from Hughes Creek were individual samples consisting of 1 pint of water per 15-minute period. Those of July 11 were gallon samples

Figure 40. Burettes with stop-cocks and a length of hose were (a) fastened to a board, (b) lowered into the stream, and (c) gravity flow provided a continuous steady deposit into a container. The amount of water could be regulated to obtain a sample of desired quantity for a set period of time.



collected in 1 hour. The results of water sampling by this method are shown in table 9.

Data collected from Hughes Creek on the latter date demonstrate the advantage of the burette. During the sampling period from 5:20 a.m. to 9:25 a.m., a total of eight dip and four burette samples were taken. Three of the burette samples contained measurable amounts of DDT while only one dip sample showed positive results. From these data it appears that several blocks of DDT passed the sampling station. The blocks were evidently separated since most dip samples showed negative results. In one instance, dip





sample number 57, showed 0.24 ppb DDT while the corresponding burette samples showed nothing. This could have occurred because a very short or small block of insecticide passed the sampling station as the dip sample was collected and the capacity of the burette was not enough to capture sufficient insecticide for detection after dilution over the entire sampling period. If this were the situation, it could probably be overcome by decreasing the sampling period duration.

As mentioned previously, burette water sampling indicates that insecticide reaching streams may drift downstream in separated blocks. Therefore, concentrations of insecticide within an individual block would, in all probability, be higher than measured for the complete sample. When such blocks do occur they can be expected to spread out and be diluted as they move downstream.

Apparently burettes are more apt to pick up contaminants, if present, from streams. Concentrations measured in a burette sample are a leveling out of variations that may actually have occurred. Thus, the findings, provide indications and trends, which are more evident by this technique than by dip sampling.

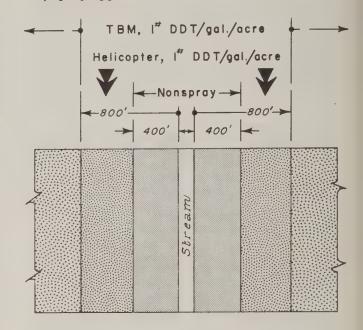
SPRAY DISTRIBUTION STUDY

Hughes Creek flows east into the North Fork of the Salmon River. The stream varies from 3 feet wide in the upper reaches to 200 feet in lower areas where there are beaver dams. Canyon slopes vary in steepness from slight up to 90 percent. Slopes, from stream to highest points of test, average 38 percent. The timber type is generally Douglas-fir with varying amounts of ponderosa pine mixed in.

The Hughes Creek drainage was used for a special test site in 1963 and again in 1964. Application rates in 1964 are illustrated in figure 41.

All spray pilots were given special ground and flight orientations to be sure they were acquainted with procedures, distances, and general terrain. Forest Service observers in observer craft were used to help guide the spray pilot, check calibration, observe any

Figure 41. This diagram shows the pattern of spray application on Hughes Creek.



errors in spraying and record area treated on an aerial photo mosaic.

Three helicopters sprayed on July 9. Spraying started at 4:35 a.m., and was completed by 9:50 a.m. The winds ranged from calm up to a maximum of 5 mph, and temperatures ranged from 45° to 65° F. There was a scattered cloud cover.

Two TBM's sprayed on July 11. Areas nearest to streams were flown first to take advantage of the best weather conditions and to insure completion of the test. This spraying also was started at daybreak, 4:35 a.m. Spraying was stopped at 10:15 a.m. because some breakup of spray pattern was observed. All areas within 1 mile of the streams were completed by this time. Weather conditions were the same as on July 9, except the sky was clear.

Comparison of Dye-cards and Filter-paper Samplers

A comparison study of dye-cards and filterpaper samplers was suggested by Mr. Kenneth Walker of Agricultural Research Service. The filter-paper samplers were made up, furnished, and later analyzed by the Agricultural Research Service. Setting out card lines was under supervision of Forest Service project personnel.

Oil-sensitive dye-cards are 4" x 5" pieces of a special grade paper coated with a red dye. When a drop of oil strikes the treated surface of the card it makes a circular spot six to eight times the diameter of the drop. The quantity of spray deposited on the card is estimated by comparing the drop pattern with the drop patterns on a series of standards. These are reproductions of cards bearing spot patterns from known quantities of spray deposit. This dye-card method is not as accurate as the more complex chemical methods for determining spray deposits, but has been accepted as adequate for aerial spray field work. It is commonly used as an indication of whether or not spray is reaching the ground, rather than as a true indication of the amount applied.

The filter-paper samplers consisted of two 4" x 5" No. 1 filter-paper sheets, stapled to a 4" x 5" chipboard backing. Laboratory analysis of each filter-paper sampler was made to give micrograms of DDT per square inch of surface.

Spray distribution was analyzed by locating eight card transects across Hughes Creek and the large tributaries. Each card transect consisted of 21 points on each side of the stream set out at a right angle to the channel. The first point in each case was on the streambank. Points were located 1 chain apart, horizontal distance, and the lines extended 20 chains up the slope. A different set of cards was used for each day's spraying. An oilsensitive dye-card and filter-paper sampler were placed at each point. Each card and sampler was secured with a large nail, and was marked to identify date, transect number, and location in transect. The cards used for the July 11 spraying were subjected to light rain showers during the night but the moisture did no apparent damage to the cards.

Both of these sampling techniques give only approximations of distribution of application as identified from sampling amounts reaching the ground. The timber canopy intercepts the major portion of the spray. Where possible, the cards were placed in canopy openings to avoid interception.

After the areas had been sprayed, the oilsensitive dye-cards and the filter-paper samplers were collected. Interpretation of dye-cards by visual comparison with the standards showed considerable variation in amount deposited. The lower limit of interpretation of amount deposited was 0.01 gallon per acre (also 0.01 pound DDT per acre).

Each filter-paper sampler was folded face to face and stapled. This procedure prevented loss of DDT from the sampler and prevented contamination of one sampler by another. Samplers were sent to the Agricultural Research Service Laboratory for analyses. Extraction, cleanup, and analytical procedures used were as follows:

Extraction

Each sampler was extracted for $1\frac{1}{2}$ hours in a Soxhlet extractor with n-hexane as the solvent. After extraction, the extract was made to 50 ml. volume and stored in screw cap glass bottles under refrigeration.

Cleanup

None required. Note: The presence of the dye from the oil dye cards did not interfere with the analysis.

Analytical

Stiff, H.A. and J.C. Castillo. A colorimetric method for the microdetermination of 2,2-bis (p-chlorophenyl) 1,1,1-trichloroethane (DDT). Science 101:440-443, 1945.

The lower limit of the analytical method for analyzing filter-paper samplers was 0.05 micrograms DDT per square inch. Below that was shown as "ND" (no detection) in the above quoted ARS report. All results were converted to "pounds per acre" in other tables in that report and the non-detectable amounts were shown as less than .001 pound per acre.

Analysis of individual transects showed

¹Agricultural Research Service mimeographed report PCY-64-21, 1964. (On file with Entomology Research Div., ARS, Yakima, Wash.)

Table 42. Comparison of dye-card and filter-paper techniques for evaluating helicopter spray distribution, south side of Hughes Creek (July 9, 1964; in pounds of DDT per acre)

	CH-1-8-5 Averages	Dye Filter Dye Filter cards paper cards paper	.01 .003 .01 .004	NS NS .01 .004	.01 .010 .014	.002	.03 .026 .03 .012	.02 .008 .25 .023	.2 .082 .20 .047	.2 .128 .20 .078		.05 .006 .25 .038	NS .004 .30 .058	.2 .043 .33 .085	.1 .003 .37 .054	NS .003 .40 .064	T .003 .40 .056	110. 20. 200. N	T .004 .15 .038	NS .004 .30 .046	NS NS .13 .029	NS NS .04 .013	100. 70. SN SN
	CH-1-7-S	Dye Filter cards paper	NS <.001	T <.001	T .001	T .002	.01 .006	.05 .004	.7 .121	.2 .024	.2 .022	.2 .052	.4 .132	.2 .030	.2 .075	.2 .028	T .006	NS .002	T <.001	NS <.001	T <.001	T <.001	NS NS
	CH-1-6-S	Dye Filter cards paper	00. 10.	.00 .006	.03 .008	.03 .014	.08 .040	1.2 .885	.2 .083	.4 .227	1.4 1.086	.4 .172	100. 70.	.01 <.001	.01 <.001	т <.001	.01 <.001	.01 <.001	.01 <.001	.00 .003	.01 .002	.02 NS	⊢ NS
Transects:	CH_1-5-S	Dye Filter cards paper	T <.001	T .005	T .001	.01 <.001	.02 .006	.2 .070	.3 .040	.5 .160	.7 .153	.1 .012	.4 .086	.7 .152	980. 7.	.1 .021	760. 2.	T <.001	1 <.001	T <.001	NS <.001	T .002	NS NS
	CH-1-4-S	Dye Filter cards paper	T .005	.01 .004	.00 10.	- NS	100. 10.	.01 NS	.01 <.001	.00 <.001	.2 .146	NS NS	.5 .163	.2 .085	.01 <.001	.7 .210	.2 .029	.1 .009	.5 .221	1.4 .737	.3 .068	.2 .057	.01 <.001
	CH-1-3-S	Dye Filter cards paper	NS .005	NS <.001	T .004	NS <.001	NS <.001	.02 <.001	T <.001	.3 .083	.02 .004	700. 6.	.3 .024	.8	1.0 .022	1.2 .095	1.3 .065	NS <.001	T .004	T .001	T .003	T .005	- NS
	CH-1-2-S	Dye Filter cards paper	T .007	T .004	T .002	T <.001	.010. 70.	.5 .062	NS .043	.00 <.001	T <.001	.1 .009	.4 .045	.5 .256	.7 .157	.3 .086	.8 .213	.05 <.001	T <.001	T <.001	T <.001	T <.001	NS NS
	CH-1-1-S	Dye Filter cards paper	.01 .005	NS .010	.01 .004	.01 .009	.02 .010	.02 .005	.01 .006	.01 .003	.01 .004	.01 .008	.01 .006	.03 .011	.2 .090	690. 2.	.4 .125	.2 .068	.7 .172	1.0 .314	.6 .128	.03 NS	.03 NS
	Feet	from	0	99	132	198	264	330	396	462	528	594	099	726	792	858	924	066	1056	1122	1188	1254	1320

Table 43. Comparison of dye-card and filter-paper techniques for evaluating helicopter spray distribution, north side of Hughes Creek (July 9, 1964; in pounds of DDT per acre)

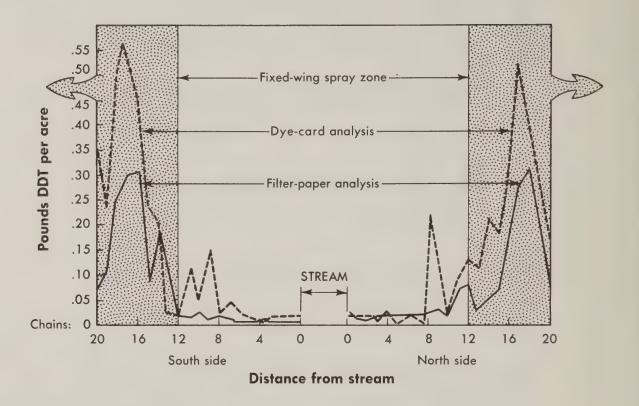
1	CH-1-1-N	-HO	CH-1-2-N	СН-	1-3-N	CH-1	CH-1-4-N	CH-	CH-1-5-N	CH-	CH-1-6-N	유	CH-1-7-N	CH-1	CH-1-8-N	Averages	nges
Dye	Filter	Dye	Filter Paper	Dye	Filter	Dye	Filter	Dye	Filter	Dye	Filter	Dye	Filter	Dye	Filter	Dye	Filter
.01	900.	H	.003	NS	<.001	1	<.001	H	<.001	10.	600.	SN	.004	NS	<.001	.01	.003
-	900.	.01	<.001	NS	<.001	NS	<.001		<.001	.01	.005	.01	.002	-	<.001	.01	100.
.0	600.	-	<.001	SX	<.001	NS	<.001	.00	100.	.01	.003	10.	<.001	—	.010	.01	.003
0.	600.	NS	<.001	-	<.001	<u>-</u>	<.001	-	<.001	.00	900.	.01	<.001	-	.004	.01	.002
.02	.012	SN	<.001	-	<.001	<u>_</u>	<.001		<.001	.02	.004	H	100.	—	.004	.00	.003
ZS.	NS	—	<.001	Ξ.	.017	~	<.001	7:	.263	.01	.002	.01	.004	.02	700.	.12	.042
60	.042	-	.005	4.	.143	NS	<.001	.7	.205	NS	NS	.01	<.001	.07	.018	.18	.059
5	.325	-	600.	7:	.189	4.	700.	7:	.269	.03	900.	.2	.004	r;	.104	.35	.123
4.	.139	H	.004	.2	.004	.2	.057	œ.	.178	1.2	.574	.2	NS	7:	.277	.46	.102
7	.332	.00	.011	.00	<.001	.07	.018	٥:	.292	1.0	.192	Ξ.	.005	1.5	.858	.53	.121
9.	.017	.01	200.	7:	620.	90.	.018	.7	.168	3.0	.537	.5	.053	1.2	.350	.85	660.
.01	900.	.02	800.	.2	.054	.03	.012	7:	.331	1.1	306	.2	.019	4.	.167	.33	.113
2	.158	.03	.015	.2	.052	.2	NS	٥;	.166	1.4	.580	7:	980.	-	.072	.47	.092
_	.024	-	800.	٥:	.026	.5	.227	9.	.136	ų.	.094	۰.	Z	9.	.176	.45	660.
80.	010.	.01	NS	.2	.034	.2	.156	7:	.172	7:	.238	9.	.027	.05	800.	.32	.081
.02	.004	.01	200.	- .	.017	.2	.077	60.	.018	1.0	.405	.2	.019	_	.004	.20	690.
.01	.005	 	.005	.03	900.	.2	.065	.2	.061	٥:	.223	ů.	NS	SZ	.002	.21	.052
-	.003	.02	SZ	.01	<.001	.5	.120	.01	900.	7:	.026	.7	.112	SX	.005	.24	.039
_	.005	.00	.018	.01	900.	ø.	.147	.03	<.001		010.	.2	.012	SX	.015	.12	.028
.01	900.	.07	<.001	_	<.001	-	.040	.02	.017	.03	.004	SZ	.033	NS	.003	.03	.013
<u>-</u>	.005	.07	NS	F	SN	.03	NS	.01	SZ	.03	NS	4.	SZ	SZ	.004	.07	.004

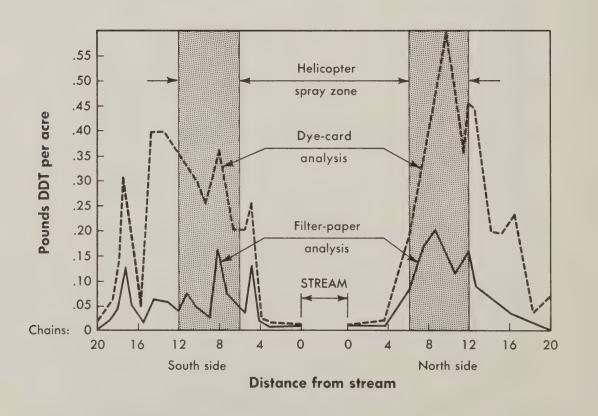
Table 44. Comparison of dye-card and filter-paper techniques for evaluating fixed-wing spray distribution, south side of Hughes Creek (July 11, 1964; in pounds of DDT per acre)

			o. I	Iransects:									
CH-2	-2-3-5	3	CH-2-4-S	동	CH-2-5-S	CH-2	CH-2-6-5	CH-2	CH-2-7-S	CH-2-8-5	-8-5	Aver	Averages
Dye	Filter	Dye	Filter	Dye	Filter	Dye	Filter paper	Dye	Filter paper	Dye	Filter paper	Dye cards	Filter
SZ S	ZZ	SX	<.001	F	<.001	.03	.005	NS	.002	NS	900.	.01	.003
S	.003	NS	.004	.00	<.001	.02	.004		<.001	;—	.002	.01	.002
—	900.	NS	.004	Ļ	<.001	\ -	.001	—	NS	F	<.001		.002
	200.	SZ	.001	.00	.002	× -	(.001		<.001		<.001	.01	.002
—	800.	NS	800.	.01	.012	-	900.		.005	-	<.001		900.
	.007	SZ	.007	90.	110.	> 10.	7.001	—	NS	.00	<.001	10.	.005
.01	.012	Z	.022	.01	Z S	.01	900.		<.001	.00	<.001	.02	800.
NS	.014	-	.049	.03	.015	.01	900.		<.001	<u>-</u>	<.001	.05	.014
H	010.	.07	.059	90.	.019	.01	.011	_	<.001	. 00.	<.001	.02	910.
—	.010	.2	.043	90.	.017	.03	010.	-	<.001	.03	<.001	.16	.015
<u>-</u>	.017	.2	080	90.	.004	.02	600.	j	<.001	90.	.015	.05	.021
—	.022	e.	.012	.03	.023	.03	.015	—	.004	90.	.017	.13	910.
NS	.030	-	.010	.03	.023	.04	.015	.01	.001	.07	.014	.02	910.
.03	909.	NS	<.001	90.	.028	.05	.015	-	700.	Ξ.	.014	.03	.112
0.	.825	—	<.001	90.	.027	.03	.025	ω.	.042	NS	NS	.20	.184
.2	.017	-	.150	.02	.030	.03	.038	4.	.122	.2	.040	.24	.063
.07	<.001	7:	.542	—	800.	SZ	890.	1.2	.761	۲.	.450	.47	.305
	<.001	1.0	.385	.7	.191	80.	SN	.7	.613	1.0	.319	.56	.302
	<.001	9.	.174	1.2	.700	SZ	.058	.2	.123	9.	.407	.49	.244
	.002	4.	.059	.2	060.	-	.047	.05	.061	.7	.340	.22	.075
	SZ.	2	SZ		SZ	0.01	.045	.03	.052	4.	SN	.39	.048

Table 45. Comparison of dye-card and filter-paper techniques for evaluating fixed-wing spray distribution, north side of Hughes Creek (July 11, 1964; in pounds of DDT per acre)

								Т	Transects:									
Feet	CH-	CH-2-1-N	CH-;	CH-2-2-N	CH-2	-3-N	CH-2-4-N	N-4-	CH	CH-2-5-N	유	CH-2-6-N	CH-2	CH-2-7-N	CH-2	CH-2-8-N	Aver	Averages
from	Dye cards	Filter paper	Dye	Filter paper	Dye	Filter paper	Dye cards	Filter paper	Dye	Filter	Dye	Filter	Dye	Filter	Dye	Filter	Dye	Filter
0	ь	3	NS	010.	_	600.	V	<.001	.01	.003	.03	NS	SZ	.047	SZ	.001	10.	.012
99		1	.01	010.		.017	V	<.001	⊢	.007	.03	<.001	SZ	SZ		.001	.01	900.
132	F	d \	—	.002		700.	V	<.001	F	.010	· 90·	<.001	-	.002	.01	.001	.01	.003
198		Α,	F	.003	.02	.026	V	<.001	-	.022	.01	.003		.002		.001	.01	.008
264		∀ :	F	.004	.01	.016		010.		.026	.01	.003	—	NS	90.	.001	.01	010.
330	NS	S	H	900.		.012	-	800.	_	.028	NS	NS		.003		.004		010.
396	SZ	0	-	600.		.015	jua .	.011		.014	90.	.003		.004	-	200.	.01	600.
462		N		.012	.01	600.		.010		.021	.01	800.		.005	-	.002	.01	010.
528			10.	200.	9.	.036		.015	—	.032	 	.010	-	.003	NS	010.	60.	.016
594			—	.002	1.7	.053	-	.015	—	.052	—	600.	—	.003	.02	.012	.22	.021
099				.003	—	.004	⊢	.016	.01	090.	90.	.012	—	700.	.00	.014	.01	.017
726			NS	600°	.01	.005	—	.015	.7	.465	-	700.	.01	800.	10.	610.	.10	.075
792	-		jav.	.004	 	900°	Н	.019	9.	.504	.01	.015	.01	.011	4.	.028	14	.084
858	7:			.003	_	900.	.02	.022	90.	.083	.01	.018	.01	.011	.2	.022	.12	.024
924	1.0			<.001	—	.011	.05	.050	.05	.029	۳.	.186	90.	.018	NS	.052	.21	.049
066	.2			<.001	F	.013	.2	890.	9.	.340	~	.050	.2	.043	.2	.054	.19	.081
1056	.2			.007	—	.014	4.	.393	7:	.370	.03	.051	1.0	.132	- .	.061	.30	.147
1122	.5		Н	.004	1.0	.641	.5	.265	4.	.162	.07	.045	1.2	.570	SN	NS	.52	.248
1188	۳.			.002	1.5	1.239	9.	.375	NS	.215	.02	.051		600.	NS	NS	.40	.315
1254	4.		-	SZ	.5	309	9:	.350	٠.5	.214	90.	.065		600.	NS	NS	.29	.189
1320	.2		.01	NS	.03	NS	.2	NS	, 6	NS	.01	.057		NS	.2	NS	.16	.057





great variability in amounts deposited and in distribution (tables 42-45). This is to be expected since timber canopy, topography, local weather conditions, and flight patterns all vary from point to point and time to time. A composite of the eight transect readings were, therefore, averaged for the various distances from the stream to give the spray distribution and amount deposited at ground level (fig. 42).

It is apparent from the data in these tables and diagrams that filter-paper samplers more reliably show the smaller quantities of DDT. The lower limit of confidence for interpreting dye-cards is 0.01 pound per acre whereas for filter-paper samplers it is 0.001 pound per acre. When very small amounts of insecticide reached the stream, they could be measured by filter-paper sampling.

There was a slight peaking of spray deposits near the stream at the canyon bottom. The exact cause is not known, although it appears to have been the settling of fine particles from both sides of the stream at the coolest or lowest level. This stacking effect was more noticeable for fixed-wing planes than for helicopter spraying.

When the fixed-wing planes were operated, there was a slight breeze from the north. This could have carried spray mist away from the north slope and onto the south slope. The graphs show more variation on the north side than on the south side of the stream.

Correction, or spread factors, are used for interpreting results on dye-cards from the set of index cards. Throughout the spray project, and for this particular comparative test, the spread factor of 5.50 was used. Results in the tables and graphs are based on this spread factor. Readings for measurable amounts were all quite high, about double the amounts found on the filter-paper samplers.

Interpretations were made for a number of dye-cards using a spread factor of 7:50. Results are not shown here, but they did indicate interpretations much closer to filter-paper sampler results than when 5.50 was used. If additional use is made of dye-cards for measuring applications of similar formulations, the 7.50 spread factor index should be used but it should also be checked again.

Figure 42. The TBM and helicopter spray distribution (the average rate by distance from the stream) is shown for Hughes Creek, 1964.

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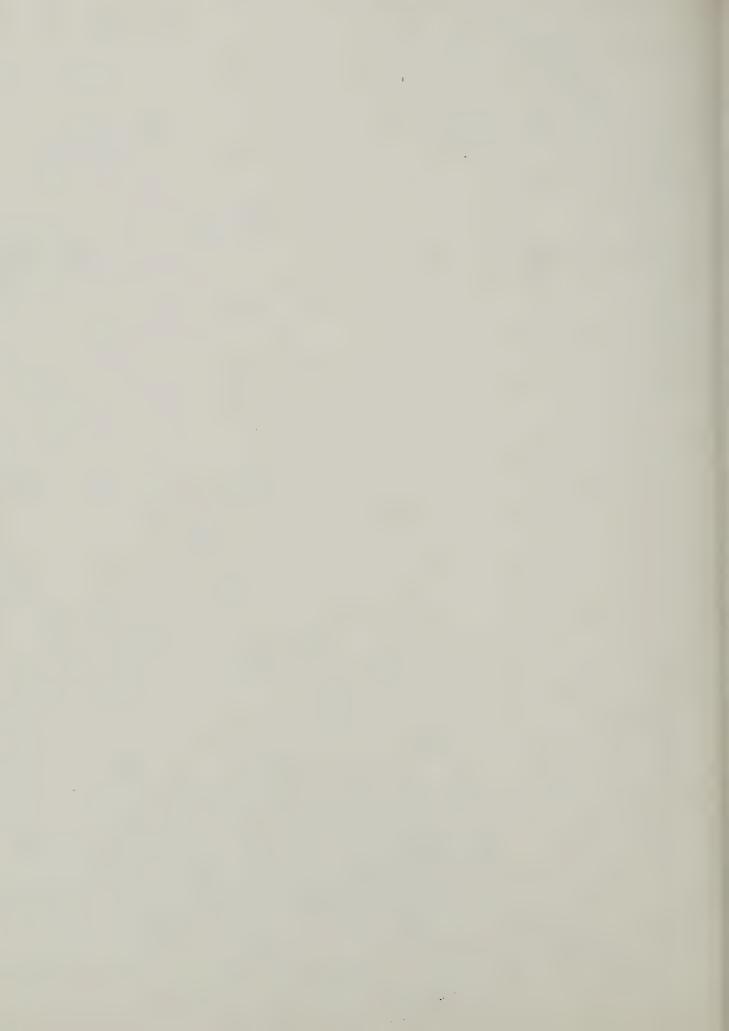
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ADDENDUM



THE EFFECTS OF A DDT SPRAY APPLICATION PROGRAM ON THE AQUATIC ORGANISMS IN HUGHES CREEK, IDAHO 1963, 1964

by

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A report prepared by the
IDAHO FISH AND GAME DEPARTMENT
In cooperation with the
UNITED STATES FOREST SERVICE
Region Four

1965

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FOREWORD

In 1963 and 1964, the United States Forest Service and Idaho Fish and Game Department conducted an evaluation of Forest Service proposed stream protection measures during aerial spraying of forest areas with DDT for the control of spruce budworm. The data and analyses of the Fish and Game Department are presented in this report.

Forest Service personnel contacted representatives of the Fish and Game Department in the spring of 1963 to inform them of plans to spray large blocks of the Salmon and Challis National Forests with DDT in the summer of 1963. These areas are important watersheds of chinook salmon (Oncorhynchus tshawytscha) and steelhead trout (Salmo gairdneri) streams in the Salmon River drainage. Forest Service personnel proposed a spray application program which they believed would provide adequate DDT spray distribution for budworm control, and cause only minimum damage to the aquatic organism populations, especially the salmon and steelhead trout. Some forest areas in the Salmon River drainage had been sprayed with DDT in past years. The effects of such spray projects on aquatic organisms had not been assessed, however. There are numerous reports in the literature of varying degrees of mortality to aquatic insects and fish as a result of spraying forests with DDT. Consequently, the recommendations of the Idaho Fish and Game Department, in 1963, were of necessity based on the results of DDT spraying programs conducted elsewhere and reported in the literature.

The only report found by the authors where significant fish or aquatic insect mortality did not occur as a result of aerial spraying with DDT was that of Todd and Jackson, 1961. The special precautions to avoid getting DDT in the streams included: (1) a strip approximately ½ mile wide on either side of the main stems of major streams was

left unsprayed; (2) DDT was applied at a rate of 0.25 pound per acre; and (3) smoke bombs were used to mark streams and identify ground wind drift conditions.

The Fish and Game Department recommended to the Forest Service that the areas in the Salmon River drainage be sprayed in a like manner. This recommendation was believed necessary because of the important salmon and steelhead resources involved and because the stream protection measures proposed by the Forest Service had not been tested.

Forest Service personnel believed that adequate spruce budworm control could not be obtained if the DDT was applied as recommended by fishery biologists of the Fish and Game Department and Forest Service. To resolve the problem, spraying in the Salmon River drainage was postponed for a year to test the spray program on a small drainage.

In 1963, the Forest Service and the Idaho Fish and Game Department conducted an evaluation of Forest Service proposed stream protection measures on Hughes Creek, a small stream located in the Salmon National Forest. The application plan specified a 100-foot wide nonspray strip and a 300-foot wide strip sprayed with a concentration of 0.5 pound DDT per acre by helicopter. A 600-foot wide strip was sprayed with a concentration of 0.5 pound DDT per acre by fixed-wing aircraft, and the remainder of the test area was sprayed with a concentration of 1 pound DDT per acre by planes.

In 1964, the Forest Service decided to spray 500,000 acres of the Salmon National Forest using stream protection measures similar to those tested in the 1963 spraying of the Hughes Creek drainage. The application plan specified a 300-foot wide nonspray strip, a 400-foot wide strip sprayed with helicopters at a rate of 0.5 pound DDT per acre, and the

remainder of the forest sprayed by planes at a rate of 1 pound DDT per acre beginning 700 feet from the stream.

Within the 1963 Hughes Creek project the spruce budworm mortality in the 0.5 pound DDT per acre areas was significantly less than the 1 pound DDT per acre spray areas. The Forest Service, therefore, desired to conduct another experimental spray project on Hughes Creek. In 1964, a spray application program designed to use a concentration of 1 pound DDT per acre was tested. The original plans for this study specified a 100-foot nonspray strip. Just previous to begin-

ning this study, monitoring work on the main 1964 spray project showed that helicopters could not spray down to within 300 feet from the stream without depositing excessive amounts of spray in the stream. Consequently, the 1964 Hughes Creek application pattern was redesigned. The nonspray strip was widened to 400 feet; the adjacent 400-foot strip was sprayed with a concentration of 1 pound DDT per acre by helicopters. The remaining area was sprayed with a concentration of 1 pound DDT per acre by fixed-wing aircraft. All formulations were at the rate of 1 gallon fuel oil carrier per acre.

ACKNOWLEDGEMENTS

The Fish and Game Department sincerely appreciates the opportunity to evaluate the DDT application program and assist in determining a program that will minimize the detrimental effects on the aquatic organisms, particularly salmon and steelhead trout. This evaluation would not have been possible without the full cooperation of the U. S. Forest Service in providing information, manpower, materials, and funds.

SUMMARY

In 1963, the U. S. Forest Service proposed spraying a large block of the Salmon River drainage for control of the spruce budworm. Original plans called for spraying up to within 100 feet of the streams with a helicopter applying 0.5 pound DDT per acre. A test area (Hughes Creek) was sprayed to determine if the spray application program proposed by the Forest Service would keep DDT out of the streams.

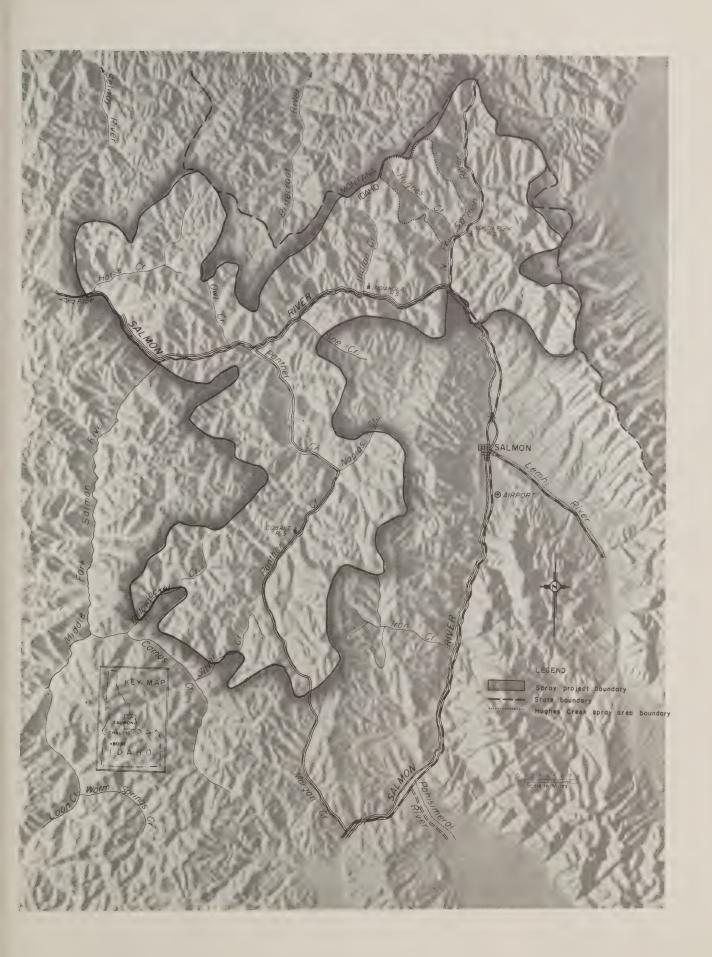
The spray distribution, as measured by a number of spray card lines, tended to be more protective of the stream than had been planned. The average width of the nonspray strip (less than 0.1 gal/A), was 300 feet rather than 100 feet as planned.

DDT caused insect and fish mortalities did occur. The more serious mortalities, however, occurred in or immediately below the mouths of unprotected tributary streams.

In 1964, Hughes Creek was sprayed again to test a revised application plan based on results of the 1963 tests. This plan called for helicopters to spray 1 pound DDT per acre to within 400 feet of all streams flowing more than 5 cubic feet per second. The spray was distributed very nearly as planned. The helicopter spray strip averaged 478 feet from the stream instead of the proposed 400 feet.

Spraying by fixed-wing, single engine aircraft caused substantially higher aquatic insect losses than the spraying by helicopters.

Figure 1. Salmon City area showing the Hughes Creek spray program test area in relation to the control stations and to the general spray, 1964.



THE TEST AREA

The Hughes Creek drainage, a tributary of the North Fork of the Salmon River, was chosen as the test area (fig. 1).

Hughes Creek is a small stream (mean flow approximately 20 cfs) with only limited natural populations of chinook salmon and steelhead trout. The steepness of the Hughes Creek drainage is representative of the main spray project area. The vegetative canopy over Hughes Creek is quite dense and representative of streams of similar size. Large streams would not have as extensive protective canopy to intercept spray. The spray area varies in altitude from 4,000 feet to 6,500 feet with a mean altitude of approximately 5,000 feet. Total acreage of the Hughes Creek spray area was approximately 16,000 acres.

Figure 2. Hughes Creek DDT spray program test area showing location of test and control stations, oil-sensitive card lines, and Scale in Miles spray area boundary, 1963. Spray area boundary Spray card line Control station Test station LEGEND T-2 C-3

THE PROPOSED APPLICATION PROGRAM

1963

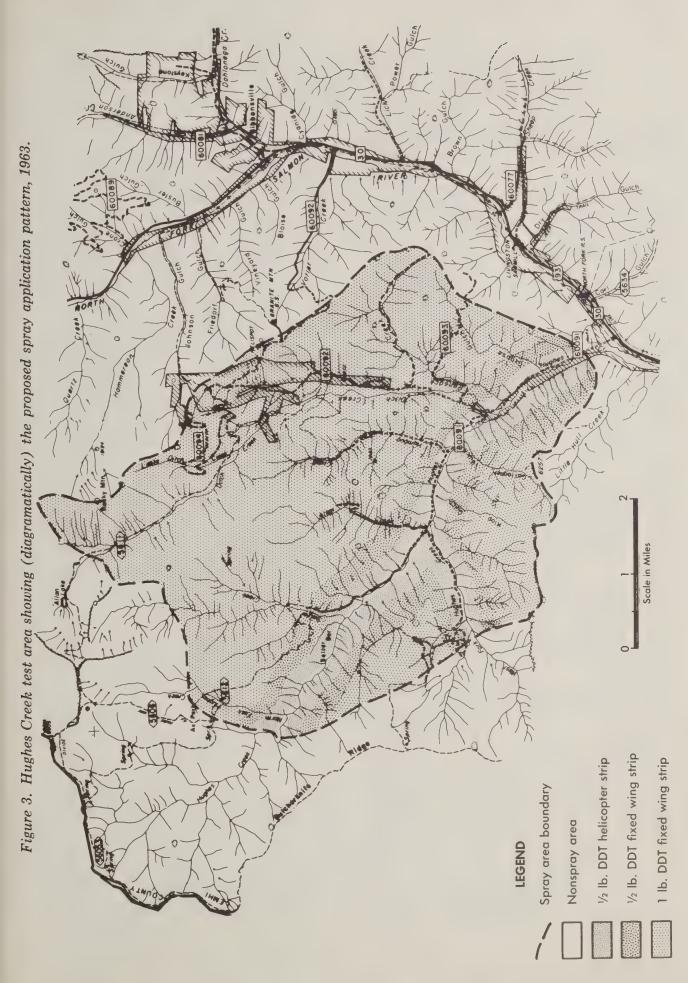
The stream protective measures to be evaluated consisted of (fig. 3): (1) an unsprayed strip approximately 100 feet wide on both sides of designated streams; (2) adjacent to the unsprayed strip, a strip 300 feet wide to be sprayed by a helicopter with a concentration of 0.5 pound DDT per acre; (3) adjacent to the 300-foot wide strip, a strip 600 feet wide to be sprayed by a TBM-type aircraft with a concentration of 0.5 pound DDT per acre; and (4) the remainder of the area to be sprayed by a fixed-wing aircraft with a concentration of 1 pound DDT per acre.

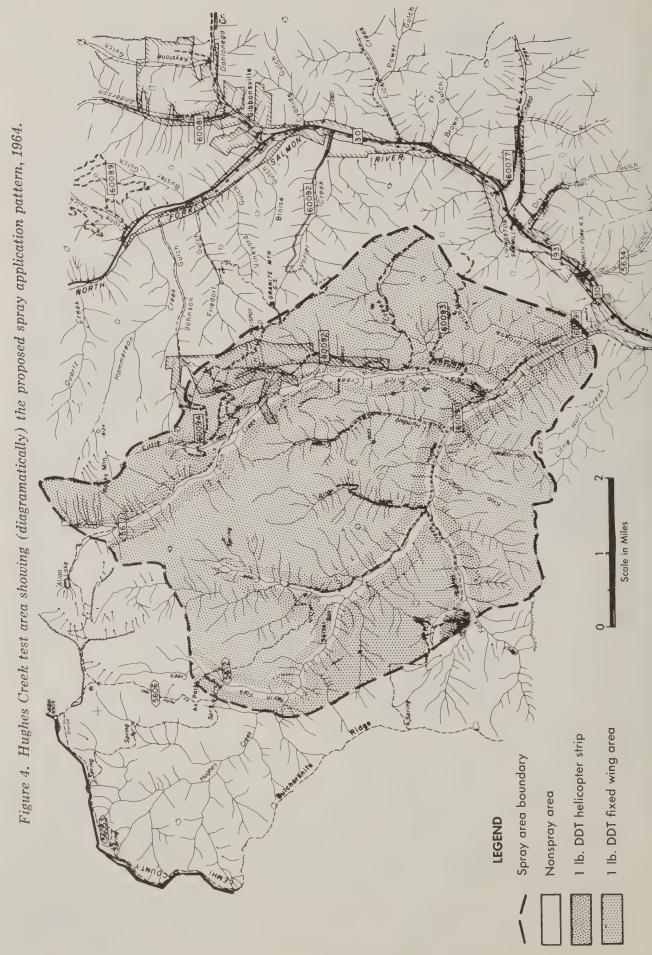
Stations T-4 and T-5 were located on Ditch Creek which received no special protective measures.

1964

The DDT spray application program for 1964 consisted of (fig. 4): (1) an unsprayed strip approximately 400 feet wide on each side of streams with a flow of 5 cfs or larger; (2) the next 400-foot strip was sprayed by helicopter with a concentration of 1 pound DDT per acre; and (3) the remaining area was sprayed by single engine plane with a concentration of 1 pound DDT per acre. Streams smaller than 5 cfs received no special protection.

Weather stations were set up in the test area to monitor wind speed and air temperature. Spraying was suspended when the wind exceeded 6 miles per hour or the air temperature 68° F.





SPRAY DISTRIBUTION

1963

Helicopter spray with 0.5 pound DDT was done on June 30. Spraying by the fixed-wing aircraft with 0.5 pound DDT was completed on July 1, and the 1 pound DDT mixture was sprayed on July 2 and 3.

Distribution of the spray materials reaching the ground near the streams was determined from oil-sensitive cards which were placed along 12 predetermined lines each day before spraying (fig. 2). Forest Service personnel devised the following system whereby each day the relative distribution of spray reaching the ground could be determined.

Five series of cards were set out on each of the 12 lines (SL-1-N, SL-1-S, ---, SL-6-S) during the 4 days of spraying.¹

Series 1 cards were put out at the first eight stations of each line to measure the distribution of the first day's spray by the helicopter. These stations were located 1 chain horizontal distance apart beginning at the stream edge.

Series 2 cards were placed at all 20 stations on each line both the first and second days to measure the spray distribution by both helicopter and TBM spraying the 0.5 pound mixture.

Series 3 cards were put out on the second morning to measure only the distribution by the TBM spraying the 0.5 pound mixture.

Series 4 cards were put out during the third morning of spraying to measure TBM distribution of the 1 pound per acre mixture adjacent to the 0.5 pound spray concentration areas.

Series 5 cards were put out each morning of spraying to measure the distribution of spray obtained by all aircraft and mixtures. The oil-sensitive cards showed that the actual spray concentrations and distribution were more protective of the stream than had been planned. (fig. 5).

Concentrations of more than 0.1 gallon spray per acre (smallest concentration readily recognizable on cards) were found on only one card line (SL-4-S) of cards placed in the proposed 100-foot nonspray strip. The average width of the strip on each side of the stream that received less than 0.1 gallon of spray per acre was 300 feet.

The 0.5 pound rate application by the TBM was not found to overlap into the proposed helicopter strip on any card lines. The average distance from the stream to the start of the 0.5 pound DDT spray by TBM was more than 800 feet rather than the proposed 400 feet. The 1 pound DDT spray rate by TBM was to have started about 15 chains from the stream. Evidence of the 1 pound DDT concentration of spray reaching the ground within 20 chains of the stream was found on only 4 of the 12 card lines (fig. 5).

1964

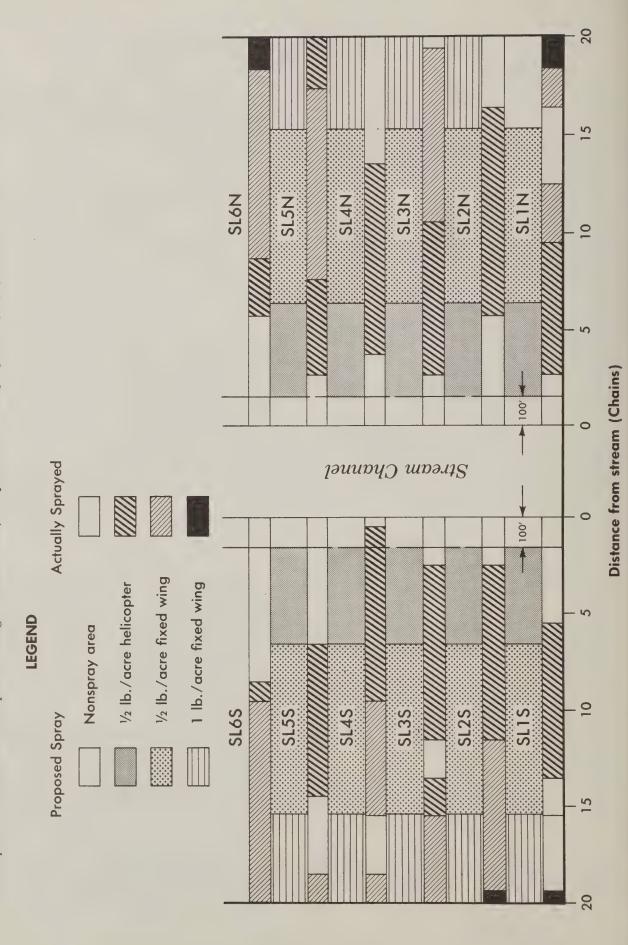
DDT spray was applied by helicopters on the strip adjacent to the nonspray strip on July 9 and 10. The remainder of the forest area up to 6,000 feet in elevation was sprayed by fixed-wing planes on July 11. Because of slower budworm development, the area above 6,000 feet elevation was not sprayed until July 16 and 17.

The distribution of spray reaching the ground near the streams was determined by placing oil-sensitive cards and filter-paper samplers on 16 predetermined lines (fig. 6) each day before spraying began. Each day, after spraying had ceased, the cards were picked up.

Two series of cards were set out during 4 of the 5 days of spraying. Spraying on the fifth day was conducted on the extreme upper

¹Each spray card was marked with symbols to designate the station number and also the side of the stream on which the line was located. The symbols "SL-1-S" mean: spray card line number 1, south side of stream.

Figure 5. Distribution of spray reaching the ground from various aircraft and DDT concentrations (0.1 lb./A or more) as determined from oil-sensitive cards placed along 12 cards lines, compared with the proposed spray pattern, 1963.

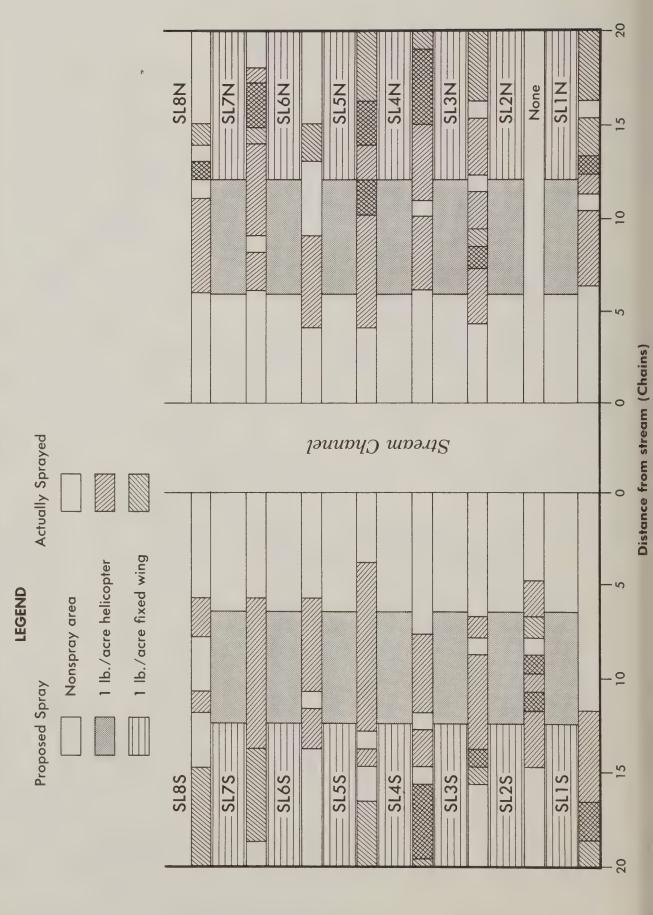


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Figure 6. Hughes Creek DDT spray program test area showing location of the test stations and oil-sensitive card lines, 1964.



Figure 7. Distribution of spray reaching the ground from various aircraft (0.1 lb./A or more) as determined from oil-sensitive cards placed along 16 card lines, compared with the proposed spray pattern, 1964.



end of Ditch Creek above any card lines so no cards were set out. Sixteen lines of stations were located and identified in the same manner as in 1963.

Series 1 cards were put out at each station of each card line on the first and second day of spraying to determine the distribution of spray from the helicopters.

Series 2 cards were placed at each station of each card line on the third and fourth day of spraying to determine the spray distribution from the fixed-wing aircraft.

As determined by using the oil-sensitive cards as indicators of distribution, the spray application was fairly close to the proposed pattern. If less than 0.1 pound DDT per acre was indicated on a card, that particular card was assumed to have not been within a spray swath. Spray in excess of 0.1 pound per acre was found on cards placed in the nonspray area on 8 of the 16 card lines. Only one of these card lines (SL-5-S) however, showed more than trace amounts of spray closer to the stream than 330 feet (fig. 7).

The average width of the nonspray strip on each side of the stream, as determined from the oil-sensitive card lines, was 478 feet rather than the planned 400 feet.

TBM aircraft spraying appeared to be overly conservative for stream protection with only 4 card lines out of 16 showing spray within 1 chain of the proposed 800-foot mark. The lower edge of spray from TBM aircraft averaged 945 feet from the stream instead of 800 feet (fig. 7).

On one card line (SL-2-N, fig. 7) no spray from the helicopter or plane was deposited within 20 chains (1,320 feet) of the stream. This was no doubt due to protection which was afforded the confluence of Ditch Creek and Hughes Creek by the planned protection widths. On another card line (SL-6-S, fig. 7) no spray from planes was deposited within 20 chains of the stream.

Spray distribution in 1964 was also evaluated by the use of 4 x 5-inch filter-paper samplers (fig. 8).

The concentration of DDT upon these papers was determined by quantitative analysis at the Agricultural Research Service Laboratory, Yakima, Washington. The lower limit of the analytical method employed was 0.001 pound of DDT per acre. According to their findings, the average distance that helicopters deposited spray (0.1 lb./A or more) from the stream was 639 feet, compared to 478 feet as shown by the oil-sensitive cards. The average distance from the stream to the edge of spray deposited by TBM aircraft was 1,122 feet compared to 945 feet as shown by the oil-sensitive card data.

The average pounds of DDT per acre for all types of application at 1-chain intervals from the stream as found on the filter-paper samplers is summarized in table 1.

According to these data, concentrations of DDT averaged higher for stations immediately adjacent to the stream than for those stations 66, 132, and 198 feet back from the stream. This suggests that some of the spray, or finer "mist," tended to settle at the lowest part of the slope and explains, in part, how some DDT got into the stream.

The average concentration of DDT reaching the ground in the helicopter 400-foot spray zone was .062 pound per acre and for the fixed-wing aircraft spray zone, it was .110 pound per acre.

It should be pointed out that some of the spray cards were possibly not hit by spray because of the "shading" effect of overhanging tree branches, tall weeds, or wind currents. Precaution was taken while placing the card stations to position them in the best available location so that any variation caused by "shading" would be minimized. The use of cards is recognized as a technique for sampling an area and is not expected to measure precisely the distribution of spray materials for the area sampled. A total miss on a card does not necessarily mean a total miss in the area sampled. A hit on a card usually denotes spray in the sampled area.

Figure 8. Distribution of spray reaching the ground from various aircraft (0.1 lb./A or more) as determined from 4 x 5-inch filterpaper samplers placed along 16 card lines, compared with the proposed spray pattern, 1964.

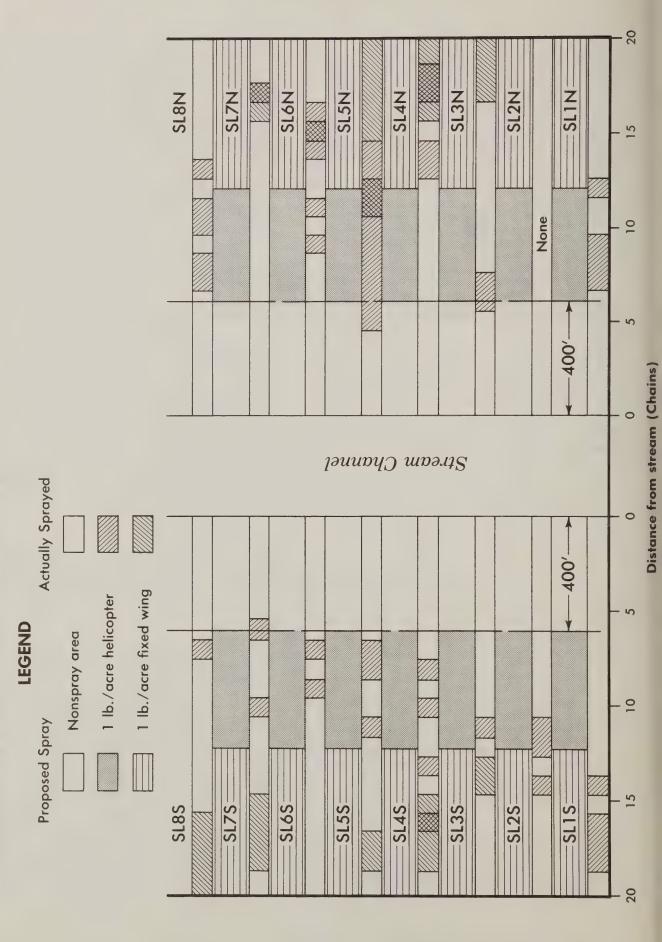


Table 1. The distribution of DDT near the stream in average pounds of DDT per acre, as determined from 4 x 5-inch filter-paper samplers¹

Chain number	Distance from	Average	Range in lb. DDT/A.	Number samples
(all transects)	stream in feet	lb. DDT/A.	Low ² High	analyzed
		Unspre	gyed	
0	0	.009	.047	16
1	66	.006	.017	15
,	132	.005	.010	18
2 3	198	.007	.026	15
4	264	.010	.040	21
5	330	.028	.263	19
6	396	.042	.205	21
Average 0 through 6	0,0	.015		
		Helico	pter	
7	462	.070	.325	25
7	528	.052	.277	23
8 9	594	.056	.292	24
	660	.052	.350	27
10	726	.078	.465	27
11	792	.066	.504	25
12 Average 7 through 12	792	.062	.504	20
Average / Introogn 12		Fixed-	wina	
	0.50	.078	.606	26
13	858	.102	.825	24
14	924	.067	.405	24
15	990		.761	23
16	1056	.158	.613	20
17	1122	.189	1.239	22
18	1188	.171	.350	21
19	1254	.082	.052	5
20	1320	.033	.032	•
Average 13 through 20		.110		
Average 0 through 20		.065		

¹Samplers were placed on perpendicular card lines from the stream at 1-chain intervals, 1964.

²One or more samples in each transect contained less than 0.001 pound of DDT/A, the lower limit of the analytical method employed.

ANALYSIS OF WATER SAMPLES FOR DDT

1963

Subsurface water samples were collected hourly during spray days at each of the test stations. The samples were dipped from the stream in 2-quart glass jars, labeled, and later analyzed for DDT concentration, by means of a gas chromatograph, at the Idaho State Department of Agriculture laboratory.

Only small quantities (.0001-.0003 ppm) of DDT were found in one sample taken at each of test stations 2, 3, and 4, and in two samples taken at each of test stations 6 and 7. The quantities of DDT found in the samples may or may not have been the maximum amount present in the stream at any one time since these were hourly-dipped samples instead of continuous samples.

From these results it is evident that if much spray materials did get into the streams, the technique did not properly sample the amount; or, if the water was adequately sampled, only minute amounts caused measurable effects as is shown later in this report.

1964

During the 1964 study, water samples were dipped from the stream at 15-minute intervals from test stations 2, 3, or 5 on each day of spraying activity. Sampling began at 4 a.m. and continued until 2 p.m.

These subsurface water samples were collected in 1-gallon tin-plated cans. The analysis of the water samples for DDT was conducted at the U.S. Agricultural Research Service Laboratory in Yakima, Washington, by means of a gas chromatograph.¹

The concentration of DDT in the water samples ranged from less than 0.2 part per billion (the lower limit of sensitivity of the method used) to a high of 162.8 parts per billion. The higher value was for a water sample taken from a slough just off Hughes Creek that had a noticeable oil slick on the water surface.

Increased DDT concentrations in the water samples did not correspond with increases in the number of insects collected in drift samples in every instance (fig. 9). At test station 2 the increases in drifting insects were quite small, as were the increases in DDT concentrations. On July 17, however, one water sample taken at 7:45 a.m. contained 4.2 ppb of DDT, but no increase in the number of drifting insects was detected.

At test station 3 on July 9, the number of drifting insects increased to some 80 insects per sample at 7 a.m., but the water samples did not exceed .44 ppb of DDT. On July 11, DDT concentrations in the stream reached a high of 2.6 ppb and drifting insects correspondingly increased to 555 insects per sample.

Water samples were taken on only one day at test station 5. On July 10, the water samples showed a high of 4.76 ppb DDT and drifting insects also increased to a high of 100 insects per sample.

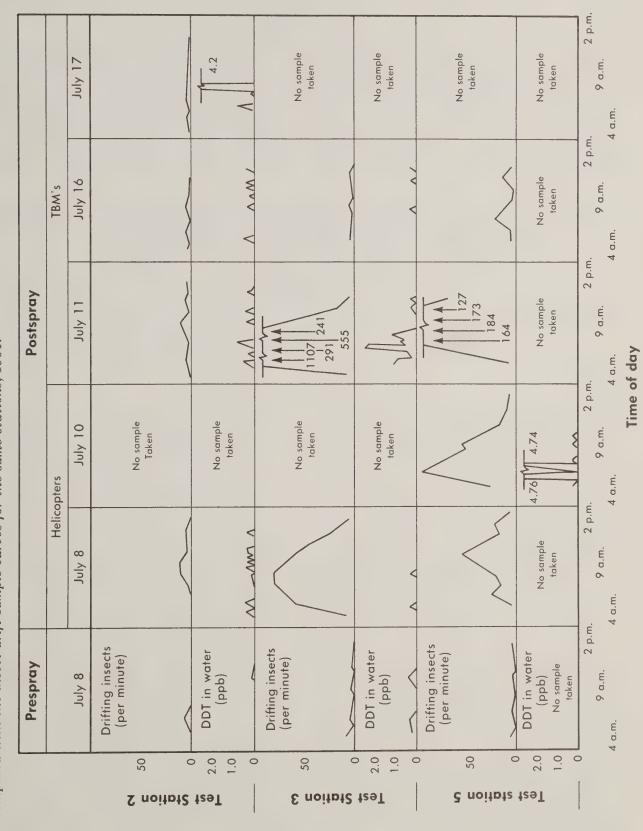
Water samples were taken at the C-2 control station on July 8. All of the eight samples taken showed less than 0.2 ppb DDT.

Personnel of the Forest Service installed a burette in Hughes Creek at the T-1 station that was calibrated to take a 1-pint water sample over each 15-minute period. On July 9, 33 of these samples were taken. On July 11, 6 hourly burrette samples were taken, as well as 29 samples that were dipped from the stream. Upon analysis, 60 of these samples contained less than 0.2 ppb DDT and one sample contained a high of 0.6 ppb DDT.²

¹ Agricultural Research Service mimeographed report PC4-64-22, 1964 (On file with Entomology Research Div., ARS, Yakima, Wash.)

²Casebeer, Robert L., Monitoring the 1964 Spruce budworm aerial spray project, 1965. See section "test use of burettes for water sampling."

Figure 9. Concentration, in ppb, of DDT in water samples taken at 15-minute intervals at test stations 2, 3, and 5. These curves are compared with the insect drift sample curves for the same stations, 1964.



MORTALITY OF FISH PLACED IN LIVE BOXES

1963

At each of the seven test and three control stations, three live boxes (18 x 30 x 24 inches) were placed in the stream. Chinook salmon fingerlings (3-4 inches in length) collected from the Lemhi River with an electric shocker were placed in one box. Steelhead trout fry

(newly emerged) from a hatching channel on the Lemhi River were placed in the second box. Hatchery-reared rainbow trout fingerlings (2-3 inches in length) from the Mackay Hatchery were placed in the third box. All of these boxes were located in pools in the stream. Wood baffles were nailed on the up-

Table 2. The number of chinook salmon fingerlings, steelhead trout fry, and hatchery-reared rainbow trout fingerlings found dead each morning during the live-box testing period, 1963 (Spraying took place July 1, 2, and 3.)

			T	est stat	lions			Con	trol stat	ions
Date	1	2	3	41	5 ¹	6	7	1	2	3
				Chi	nook salı	mon fing	gerlings			
June 29	1	2	1	2	2	3	2	0	4	2
June 30	4	2	9	7		4	1	12	5	4
July 1	0	0	0	1		0	0	1	1	0
July 2	0	0	0	1		0	1	0	0	0
July 3	1	1	1	0		1	3	0	1	0
July 4	0	0	0	5		2	2	0	0	0
July 5	0	0	0	1		0	0	1	0	0
July 6	0	0	0	0		0	0	0	0	0
July 7	0	1	0	0		0	0	0	0	1
July 8	0	0	0	0		0	0	0	0	0
July 9	3	0	0	1		0	0	1	2	0
Total number										
found dead	9	6	11	18		10	9	15	13	7
Live fish placed										Ť
in box	51	52	51	52		53	52	50	54	52
Percent mortality	17.6	11.5	21.6	34.6		18.9	17.3	30.0	24.0	13.5
					Steelhea	id trout	frv			
1 00	^	3	0			3				
June 29	0	,	0	0	0		0	0	0	0
lune 30	0		0	0	0		0	0	0	0
luly 1	0		0	0	0		0	0	0	0
luly 2	0		0	0	0		0	0	0	0
July 3	0		0	0	0		0	0	0	0
July 4	0		0	0	0		0	0	0	0
July 5	0		0	0	0		0	0	0	0
uly 6	0		0	0	0		0	0	0	0
July 7	0		0	0	0		0	0	0	0
uly 8	0		0	0	0		0	0	0	0
uly 9			2	15	7		11	2	5	4
otal number										
found dead	1		2	15	7		11	2	5	4
ive fish placed										
in box	95		98	98	100		94	100	98	96
Percent mortality	1.1		2.0	15.3	7.0		11.7	2.0	5.1	4.2

Table 2. (continued)

	Test stations							Con	trol static	ons
Date	1	2	3	41	5 ¹	6	7	1	2	3
			Hate	chery-re	ared ra	inbow 1	trout finge	rlings		
June 29	0	0	0	0	0	0	0	0	0	0
June 30	0	0	0	1	0	0	0	0	1	0
July 1	0	0	0	0	0	0	1	0	0	0
July 2	0	0	0	0	0	0	0	0	0	0
July 3	0	1	0	0	0	0	1	0	0	0
July 4	0	0	0	0	1	0	0	0	1	0
July 5	0	0	0	1	0	0	0	0	0	0
July 6	0	0	0	1	0	0	0	0	0	0
July 7	2	2	2	0	0	0	0	0	0	1
July 8	0	0	0	0	0	0	0	0	0	0
July 9	0	0	0	0	0	0	0	0	0	0
Total number found dead	2	3	2	3	1	0	2	0	2	1
		3	2	9	'		_		_	
Live fish placed in box	49	50	50	50	50	50	51	49	48	50
Percent mortality	4.08	6	4	3	2	0	3.92	0	4.17	2

¹These stations were located on a stream that received no special protection.

stream sides of the boxes to reduce water currents which might fatigue the fish. In addition, large rocks were placed in each box to provide cover and quiet water areas.

The boxes were checked each day for dead fish during the period June 29 through July 9. During the days spray was being applied (July 1, 2, and 3), the boxes were checked before spraying began. The number of dead fish found in each box each morning is listed in table 2.

The statistical test used to determine if the mortality rates which occurred at any of the stations were unexpectedly high, was the heterogeneity of the Chi-square test. A contingency table was first set up to determine if any significantly different mortality rates were present at any of the test or control stations. If the calculated value of Chi-square and its associated probability lead to the decision that the mortality which occurred at one or more of the stations was significantly different from what we would normally expect, then additional contingency tables were set up to determine at which stations the mortalities were different.

The mortalities which occurred at the control stations would theoretically represent the fish losses expected in the absence of the effects of the spray treatment.

A contingency table was set up to determine if the mortalities which occurred at the control stations were significantly different from one another. If there were no significant differences in the level of mortalities which occurred at the control stations, then the combined mortality rate for all the control stations was used as the basis for comparison of the mortalities which occurred at the test stations.

The mortalities which occurred at the test stations were the result of expected losses, the same as at the control stations, plus the losses, if any, due to the effects of the spray program. The treatment effects from the spray program were undoubtedly different for most test stations due to station locations and variable amount of DDT spray entering the streams above each test station. Thus, each test station theoretically received a different treatment from the spray application.

²No chinook salmon were placed at test station 5.

³Steelhead fry were lost from the live boxes at test stations 2 and 6.

Chinook salmon. The mortality of chinook salmon in the live boxes was higher than for either of the other two fishes. This higher mortality was undoubtedly a result of the salmon being collected with an electric shocker and perhaps their being less able to withstand the stresses of handling. Data in table

2 show that the collecting and handling mortality took place during the first 2 days after the fish were collected and placed in live boxes. Apparently most of the handling mortality had taken place before spraying began on June 30, as few dead fish were found the morning of July 1.

Table 3. Contingency tables for chinook salmon live-box tests listing the number remaining alive at the end of the test, and the number dying after June 30, 1963 (Handling mortality which occurred the first 2 days has been eliminated)

	Test stations						Control stations			
	T-1	T-2	T-3	T-41	T-6	T-7	C-1	C-2	C-3	_ Totals
Alive Dead	42	46 2	40 1	34 9	43	43 6	3 <i>5</i> 3	41 4	45 1	369 33
Total Percent	46	48	41	43	46	49	38	45	46	402
dead	8.70	4.17	2.44	20.93	6.52	12.24	7.89	8.89	2.17	8.21

 $x^2 = 15.58$ (8 d.f.) Probability of larger $x^2 = 0.05$

Between controls						
	C-1	C-2	C-3	Totals		
Alive Dead	3 <i>5</i> 3	41 4	45 1	121		
Total Percent	38	45	46	129		
dead	7.89	8.89	2.17	6.20		
	$x^2 =$	2.03 (2	d.f.)			

Probability of larger $x^2 = 0.39$

	Test station		
	T-7	C-1 - C-3	Totals
Alive	43	121	164
Dead	6	8	14
Total Percent	49	129	178
dead	12.24	6.20	7.87
	$x^{2} = 1$	1.05 (1 d.f.)	

Probability of larger $x^2 = 0.33$

Tost	station	4	VOTELLE	contro	اد
lesi	Signon	-44	versus	contro	I S

	T-4	C-1 - C-3	Totals
Alive Dead	34 9	121 8	1 <i>55</i> 1 <i>7</i>
Total Percent	43	129	172
dead	20.93	6.20	9.88

 $x_{\rm c}^2 = 6.29 \, (1 \, \rm d.f.)$

Probability of larger $x^2 = 0.013$

 $^{^{\}scriptscriptstyle 1}This$ station was located on a stream that received no special protection.

Because of the large handling mortality, the effects, if any, of the DDT on salmon would be confounded unless the handling mortality is eliminated from the analysis. The collecting and handling mortality which occurred the first 2 days (prespray) was eliminated from consideration by subtracting the losses from both the test and control stations. With the collecting and handling mortality subtracted from the data, the percent mortalities are as listed in table 3.

The x² value for the upper contingency table in table 3 is 15.58 with 8 degrees of

freedom. The probability of obtaining a larger x^2 value is only 5 chances out of 100; thus, we would reject the hypothesis that the mortality was the same at all stations.

The x² value for the test between controls was not unusually large as we would expect to find a larger one 39 times out of 100. Thus, we did not reject the hypothesis that the mortality between the various control stations was the same.

The percent mortalities at test stations 4 and 7 were the largest among the test stations. Test station T-4 was on the lower end

Table 4. Contingency tables for steelhead trout live-box tests, listing the number remaining alive at the end of the tests, and the number dying after June 28, 1963

		Test stations					Control stations		
	T-1	T-3	T-41	T-5 ¹	T-7	C-1	C-2	C-3	_ Totals
Alive Dead	94 1	96 2	83 1 <i>5</i>	93 7	83 11	98 2	93 5	92 4	732 47
Totals Percent	95	98	98	100	94	100	98 5.10	96 4.17	779 6.03
dead	1.05	2.04	15.31	7.00	11.70				

 $x^2 = 30.93$ (7 d.f.) Probability of larger $x^2 = less than 0.005$

Between controls							
	C-1	C-2	C-3	Totals			
Alive Dead	98 2	93 5	92 4	283 11			
Totals Percent	100	98	96	294			
dead	2.00 x ² =	5.10 1.40 (2	4.17 d.f.)	3.74			

Probability of larger $x^2 = 0.50$

	Test station 5	versus controls C-1 - C-3	Totals
Alive Dead	93	283	376 18
Totals	100	294	394
Percent dead	7.00	3.74	4.57
		15 (1 d.f.) larger x ² = 0.3	0

Test station 7 versus controls

	T-7	C-1 - C-3	Totals
Alive Dead	83 11	283 11	366 22
Totals Percent	94	294	388
dead	11.70	3.74	5.67

 $x^{2}_{c} = 7.02 (1 d.f.)$

Probability of larger $x^2 = 0.008$

¹These stations were located on streams that received no special protection.

of an unprotected stream. Test station T-7 was on Hughes Creek just below Salzer Creek which was an unprotected stream. A 2 x 2 contingency table was set up to test if the mortality at either of these stations was different from that at the control stations.

The corrected (for continuity) x^2 value for the test between T-7 and the control stations was 1.05, with a larger x^2 value to be expected 33 times out of 100. With that large a risk, we could not reject the hypothesis that the mortalities were the same. In other words, we are not saying the mortality rates were the same, but that they are not sufficiently different to be statistically significant.

The corrected x^2 value for the test between T-4 and the controls was 6.29, and we could expect a larger value only 13 times in 1,000. Thus, we reject the hypothesis that the mortality at test station 4 (T-4) was the same as that at the control stations. Thus, it would

appear that the spray program caused some significant chinook salmon mortality at test station 4.

Steelhead trout. As will be noted in table 2, all the dead steelhead trout fry were found when the boxes were removed from the stream at the end of the test. These small fish were hidden beneath the rocks placed in the boxes. Because the dead fish were not found prior to the end of the test, immediate mortality due to handling, if any, could not be eliminated from the analysis.

A contingency table of the number of live and dead steelhead trout found in the live-boxes at the end of the test is found in table 4. This contingency table was broken down to determine if the mortalities at any of the test stations were significantly different from those at the control stations.

In the x^2 test of mortalities between the control stations, the x^2 value was 1.40, a value

Table 5. Contingency tables for hatchery-reared rainbow trout live-box tests listing the number remaining alive at the end of the test and the number dying after June 28, 1963

			T	est static	ns				Contro	l station	15
	T-1	T-2	T-3	T-41	T-51	T-6	T-7	C-1	C-2	C-3	Totals
Alive Dead	47 2	47 3	48 2	47 3	49 1	50 0	49 2	49 0	46 2	49 1	481 16
Total Percent	49	50	50	50	50	50	51	49	48	50	497
dead	4.08	6.00	4.00	6.00	2.00	0.00	3.92	0.00	4.17	2.00	3.22

 $x^2 = 6.68$ (9 d.f.) Probability of larger $x^2 = 0.67$

Test station 2 versus 4 versus control stations

	T-2	T-41	C-1 - C-3	Totals
Alive Dead	47 3	47 3	144	238
Total Percent	50	50	147	247
dead	6.00	6.00	2.04	3.64

 $x^2 = 2.67 (2 d.f.)$

Probability of larger $x^2 = 0.27$

¹These stations located on streams that received no special protection.

that would be exceeded about half of the time. Thus, we did not reject the hypothesis that the steelhead trout mortalities at the various control stations were the same.

The corrected x² value in the test between test station 7 and the control stations was 7.02, a number that would be exceeded only about 8 times in 1,000. Thus, we rejected the hypothesis that the mortality at test station 7 was the same as those at the control stations. Since the percent dead at test station 4 was even larger than at test station 7, the x² value would be even larger and thus the mortalities at test station 4 were also significantly larger than at the control stations. As indicated previously, T-4 was on an unprotected stream and T-7 just below an unprotected stream.

Hatchery-reared rainbow trout. The mortalities of hatchery-reared rainbow trout fingerlings did not differ significantly between the test and control stations (table 5).

The lack of DDT-caused mortality among the rainbow trout is not surprising in view of the small amount of mortality occurring to the chinook salmon fingerlings and steelhead fry. Trout are less sensitive to DDT than salmon, and fingerlings are less sensitive than fry (Gagnon 1958, and Hatch 1957). The concentration of DDT in certain sections of the streams was apparently sufficient to kill small numbers of chinook salmon and steelhead trout fry but not high enough to kill rainbow trout fingerlings.

1964

Three live-boxes were placed in the stream at each of the five test and two control stations in 1964.¹

Chinook salmon fingerlings (3-4 inches) were placed in one, steelhead trout fry were placed in one, and hatchery-reared rainbow trout fingerlings were placed in the third box.

The boxes were checked each morning prior to spraying and the number of dead fish observed was recorded (table 6). The boxes were located in pools in the stream and wood baffles were used to reduce water currents which might fatigue the fish. The fish were placed in the live boxes up to a week prior to the start of spraying so that most losses due to handling would have occurred prior to the beginning of the test.

The statistical techniques used to determine if losses at the test stations were significantly different from the losses at the control stations in 1964 were the same as those used in 1963.

Chinook salmon. The percentage of fish dying in the live-boxes at the control stations was relatively high during 1964 (22 percent). A Chi-square (x^2) test indicates that the mortality rate for chinook salmon at test station 2, the test station with the largest losses, was not significantly different from the losses which occurred at the control stations (table 7).

Steelhead trout fry. The mortality of steelhead trout fry in the live-box at test station 2 was significantly larger than the losses at the control station (table 8). The losses at the remainder of the test stations were less than or not significantly larger than the mortality which occurred at the control stations.

The higher than expected losses of steelhead trout fry at test station 2 may have been due to the effects of DDT spray entering the stream. The mortality of chinook salmon and rainbow trout fingerlings at this station were no higher than at the control stations, however. There is no additional evidence which indicates the higher losses of steelhead fry were due to DDT.

Rainbow trout fingerlings. The mortalities of rainbow trout fingerlings at test stations 1, 3, and 5 were all significantly higher than the very low mortality rates at the control stations. The losses at test stations 1 and 3, even though significantly higher than the losses at the control stations, were relatively small (table 9).

The mortality of rainbow trout fingerlings at test station 5 was considerably higher than at the control stations and may have been due to DDT. The mortality rates of chinook salmon fingerlings and steelhead trout fry at test

¹The station numbers in 1964 do not correspond to those of 1963. To compare similar stations, see figures 2 and 6.

Table 6. The number of chinook salmon fingerlings, steelhead trout fry, and hatcheryreared rainbow trout fingerlings found dead each morning during the Hughes Creek DDT spray project, 1964¹

			Test station	S	e.	Control	stations
Date	1	2	3	4	5	1	2
			Chinook	salmon fir	ngerlings		
July 9	2	0	0	0	0	7	0
July 10		0		_	0	0	0
July 11		0	0	0	0	0	0
July 12		0	0	0	1	0	0
July 13		0	0	0	0	0	0
July 16			1	0	2	0	0
July 21		3	0	0	0	0	0
July 25		12	4	3	10	4	11
Total number			-		10		
found dead		15	5	3	13	11	11
Live fish placed		50	50	50	50	50	50
in box		50 30	50 10	50	50 26	50 22	50 22
Percent mortality		30	10	6	20	22	22
			Stee	lhead trout	: fry		
July 9	1	0	1	0	4	2	
July 10		Ö	<u> </u>		ī		
July 11	0	Ö	1	0	·		
July 12	0	0	0	0			
July 13	0	0	0	0			
July 16	0	1	MINISTERN .	1			
July 21	1	12	6	6	10		10
July 25	4	31	9	11	6		10
Total number							
found dead	6	44	1 <i>7</i>	18	21		20
Live fish placed							
_ in box	103	100	101	100	96		110
Percent mortality	5.82	44	16.83	18	21.87		18.18
			Dainhau	. to a cot fine			
110	0	^		v trout fing		•	
July 9	0	0	0	0	0	0	0
July 10	0	0	0	0	0	0	0
July 11	0	0	0	0	0	0	0
July 13	0	0	0	0	0	0	0
July 16	ő	Ö	0	0	0	0	0
July 21	ő	Ö	Ö	Ö	ő	Ö	0
July 25	7	Ō	5	0	23	Ö	ĭ
Total number							
found dead	7	0	5	0	23	0	1
Live fish placed							•
in box	100	100	99	100	99	100	100
Percent mortality	7	0	5.05	0	23.23	0	1

¹Spraying on Hughes Creek was conducted July 9, 10, 11, 16, and 17.

²Fish were lost from live boxes.

Table 7. Contingency tables for chinook salmon fingerlings placed in live-boxes listing the number remaining alive at the end of the tests, 1964

			Test station	15			Control static	ns
	T-1	T-2	T-3	T-4	T-5	C-1	C-2	Totals
Alive Dead	1	35 15	45 5	47 3	37 13	39 11	39 11	242 58
Total Percent		50	50	50	50	50	50	300
dead		30.00	10.00	6.00	26.00	22.00	22.00	19.33

 $x^2 = 14.04$ Probability of larger $x^2 = 0.02$

T-2	Control	Total
35	78	113
15	22	37
50	100	150
30.00	22.00	24.67
$x^2 = 1.14$	Probability of larger x	$^{2} = 0.30$

¹Fish were lost from live box.

Table 8. Contingency tables for steelhead trout fry place in live-boxes listing the number remaining alive at the end of the tests, 1964

			Test statio	ns		•	Control static	ons
	T-1	T-2	T-3	T-4	T-5	C-1	C-2	Totals
Alive Dead	97 6	56 44	84 1 <i>7</i>	82 18	75 21	1	90 20	484 126
Total Percent	103	100	101	100	96		110	610
dead	5.82	44.00	16.83	18.00	21.87		18.18	20.65

 $x^2 = 48.97$ (5 d.f.) Probability of larger $x^2 = <.005$

T-2	Control	Total
56	90	146
44	20	
100 44.00	110 18.18	210 30.48

 $x^2 = 16.46$ (1 d.f.) Probability of larger $x^2 = <.005$

¹Fish were lost from live box.

station 5 were similar to the rate for rainbow trout fingerling, but not significantly different from their controls, however. Based on reports in the literature and the results of tests from last year, if the losses of rainbow trout were due primarily to DDT, the losses of chinook

salmon and steelhead trout should have been higher than that observed. Chinook salmon fingerlings and steelhead trout fry appeared to be more sensitive to DDT than rainbow trout fingerlings in the 1963 Hughes Creek tests.

Table 9. Contingency tables for hatchery-reared rainbow trout fingerlings placed in live-boxes listing the number remaining alive at the end of the tests, 1964

		1	Test stations				Control stati	ons
	T-1	T-2	T-3	T-4	T-5	C-1	C-2	Totals
Alive Dead	93 7	100	94 5	100	76 23	100 0	99 1	662 36
Total Percent	100	100	99	100	99	100	100	698
dead	7	0	5.05	0	23.23	0	1	5.16

 $x^2 = 86.67$ (6 d.f.) Probability of larger $x^2 = <.005$

T-3	Controls	Totals
94	199	293
5	1	6
99	200	299
5.05	0.50	2.01

 $x^2 = 7.2$ (1 d.f.) Probability of larger $x^2 = <.01$

ANALYSIS OF FISH FOR DDT

1964

Samples of fish held in the live boxes were taken before spraying commenced, during the spraying activity, and after spraying terminated. All fish dying during the study were collected and also submitted to the laboratory for DDT analysis.

These fish were placed in clear plastic bags, labeled, and frozen until time of shipment to the laboratory. Upon shipping, the plastic bags containing the samples were placed in an insulated metal can which was packed with dry ice.

Unfortunately, those fish dying during the test could not be analyzed by the laboratory due to the small size of the samples. (We have since been notified by the lab that samples

smaller than 5 grams cannot be accurately analyzed.)

The DDT concentrations presented in table 10 are for composite samples of ten fish or more of the designated species.

Prespray samples of rainbow trout and chinook salmon fingerlings contained a mean concentration of .045 and .042 ppm DDT in the control and test stations, respectively (table 10). Postspray steelhead fry from the control stations showed a mean concentration of .125 ppm DDT. Postspray steelhead fry from the test stations, however, showed a mean concentration of .292 ppm.

It is unfortunate that the data are so limiting that prespray-postspray comparisons cannot be made between fish of the same species.

Table 10. The concentration of DDT, ppm, found in prespray and postspray rainbow trout fingerlings, chinook salmon fingerlings, and steelhead fry that were taken from test and control stations in the Hughes Creek study, 1964¹

			DDT concent	tration (ppm)
Date	Station	Species	Prespray	Postspray
	(Control)			
7-8-64	C-1	rainbow	.05	
7-8-64	C-2	rainbow	.04	
Mean			.045	
7-25-64	C-1	steelhead		.18
7-25-64	C-2	steelhead		07
Mean				.125
	(Test)			
7-8-64	T-2	rainbow	.09	
7-8-64	T-3	rginbow	.04	
7-8-64	T-3	salmon	.01	
7-8-64	T-4	rainbow	.03	
Mean			.042	
7-25-64	T-1	steelhead		.50
7-25-64	T-2	steelhead		.28
7-25-64	T-3	steelhead		.29
7-25-64	T-4	steelhead		.25
7-25-64	T-5	steelhead		.14
Mean				.292

¹Many more samples were taken during the study and sent to the lab for analysis but some of the samples were not large enough to be analyzed or were discarded for other reasons.

SAMPLING OF STREAM BOTTOM INSECTS

1963

Near each test station 1, 2, 4, and 7, and each control station, a large riffle was selected for sampling the stream bottom insects before and after spraying. A riffle area approximately 8 feet wide by 16 feet long was divided into 40 blocks. Ten randomly selected 2-square-foot samples were taken from each riffle 2 days before spraying, 2 days after spraying, 1 month after spraying, and 3 months after spraying. Two hundred eighty bottom samples were taken.

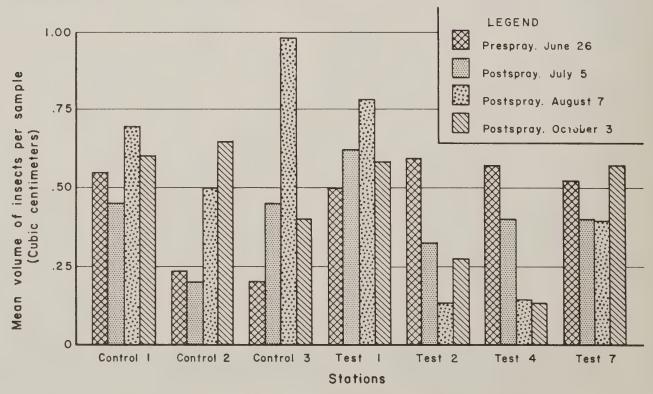
Because of the large amount of variability associated with the sampling of stream bottom insects, sampling was purposely restricted to what appeared to be a rather homogenous riffle area composed of small rubble and gravel in an effort to reduce the variability. We realized that by restricting our sampling to a selected type of stream bottom, inference from

the data would be more or less restricted to that bottom type. If we were to find significant changes in the number of organisms inhabiting the type bottom we sampled, however, it could be assumed, without too much risk, that a change in number had taken place in the other bottom types in the stream.

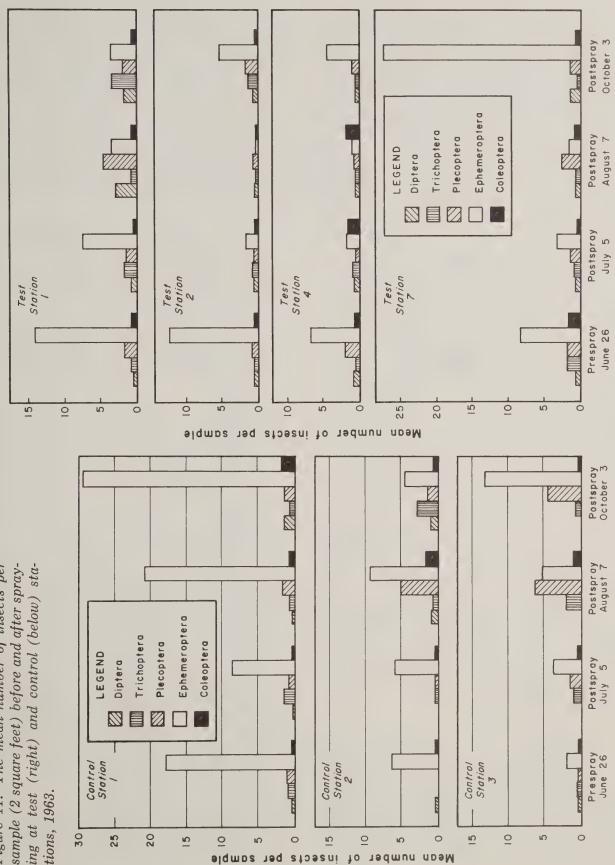
A circular frame (2-square-foot sample) covered with window screen (14 meshes per inch) was used to collect the insects dislodged from the stream bottom. The samples were placed in glass vials and preserved for analysis at a later date. The total volume and number of insects by order was determined for each sample.

At the three control stations, the mean volume of insects per sample appears to have remained fairly constant (control station 1) or to have increased (control stations 2 and 3) between the first and last sampling dates (fig. 10).

Figure 10. The mean volume of insects per bottom sample (2 square feet) for 10 samples collected on each of four different dates at each station in the test area, 1963.



5 sample (2 square feet) before and after spraying at test (right) and control (below) sta-Figure 11. The mean number of insects per tions, 1963.



At test station 1 (North Fork downstream from mouth of Hughes Creek) there was no apparent decrease in the mean volume of insects per sample. At test stations 2, 4, and 7, there appears to have been a reduction in the mean volume of insects per sample immediately after spraying. Some recovery by October was evidenced at stations 2 and 7.

In an effort to determine if any of the increases or decreases in mean volumes were due to something other than chance variation, the 95 percent confidence intervals were computed for most mean volumes. Comparisons of pre- and postspray data were limited to within station comparisons.

Even with the restricted sampling areas, the variability in the samples was quite large and most confidence intervals were plus or minus 30 percent or more of the mean. Therefore, only large differences in mean volumes could be detected as significant. Of special interest is the fact that the mean volumes for samples collected August 7 and October 3, at test stations 2 and 4, were significantly lower than the mean volumes of samples collected before spraying. The apparent reduction at station 7 was not significant. Again, it should be noted that station 4 was on an unprotected stream and station 7 was just below the mouth of an unprotected stream.

Analysis of the bottom samples by numbers of organisms of each order indicated that the mayflies (*Ephemeroptera*) were the most abundant group in the samples from all stations (fig. 11).

Mayflies also were the most affected insects at test stations where insect mortality occurred. The number apparently decreased at all test stations after spraying, with varying recovery in numbers by October.

1964

Aquatic insects from the stream bottom were collected, preserved, and analyzed in 1964 in the same manner as that of 1963. Some stations were numbered differently, however. In 1963, the test stations numbered 2, 4, 3, and 7 were numbered 1, 2, 3, and 5, respectively, in 1964. The control stations in

1964 were both in different locations than those of 1963. Because the general spray project encompassed the entire North Fork drainage in 1964, the control stations were located some 45 miles from the study area (fig. 1).

In 1964, the first postspray insect bottom samples were taken 8 days after spraying terminated, compared to 2 days after spraying in 1963. The additional time lag before sampling was planned so the collection of dead insects in the bottom samples would be minimized.

At control station 1, the trend of the *mean* volume of insects per sample throughout the summer appears to typify the trend for a normal stream. It increases sharply during the early summer, then recedes during the late summer and early fall (fig. 12).

Control station 2 exhibited unusually high volumes of bottom insects during the first three sampling periods, but each succeeding sample was noticeably less than the prespray sample.

Test stations 1 and 2 show decreases in the mean volume of insects per sample in the postspray samples of July 24 and August 19, with some recovery by October 19.

The 95 percent confidence intervals were computed for the mean volumes of insects per sample to determine if the increases or decreases were due to something other than chance variation. The prespray and postspray data were compared only within the same station. Since the confidence limits overlapped between each sampling period for the test stations, the decline noted in the quantity of bottom insects for test stations 1 and 2 could have been due to chance variation.

By separating the bottom samples into orders and calculating the mean number of insects per sample, it was apparent that mayflies (*Ephemeroptera*) and stoneflies (*Plecoptera*) were the two most prominent groups (fig. 13).

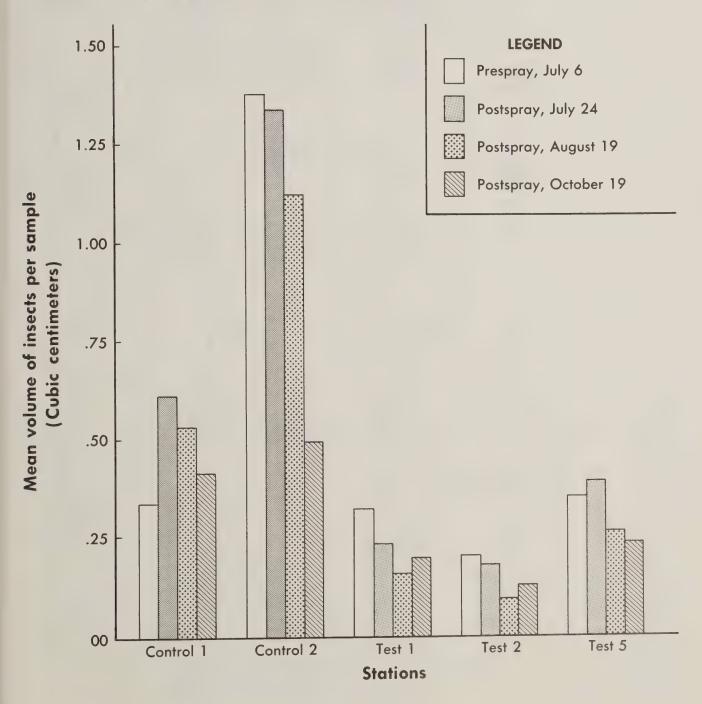
At all stations the stoneflies showed some increase in population numbers as the season progressed. In contrast, the mayfly population decreased at each station except T-5, where the first postspray sample was higher than the prespray sample. After the first

postspray samples, however, it too showed a steady decline.

By comparing the mean volume of insects per sample for 1963 with the mean volumes for 1964, it appears that the insect population in Hughes Creek underwent some change (fig. 14).

This change is most apparent by comparing the prespray volumes of the test stations. In 1963, the prespray volume averaged some 55 cc per sample while test station in 1964 averaged only 30 cc per sample. The reduction in mean volume of insects per sample immediately after spraying in 1963 was much

Figure 12. The mean volume of insects per bottom sample (2 square feet) for 10 samples collected on each of four different dates at each station, 1964.



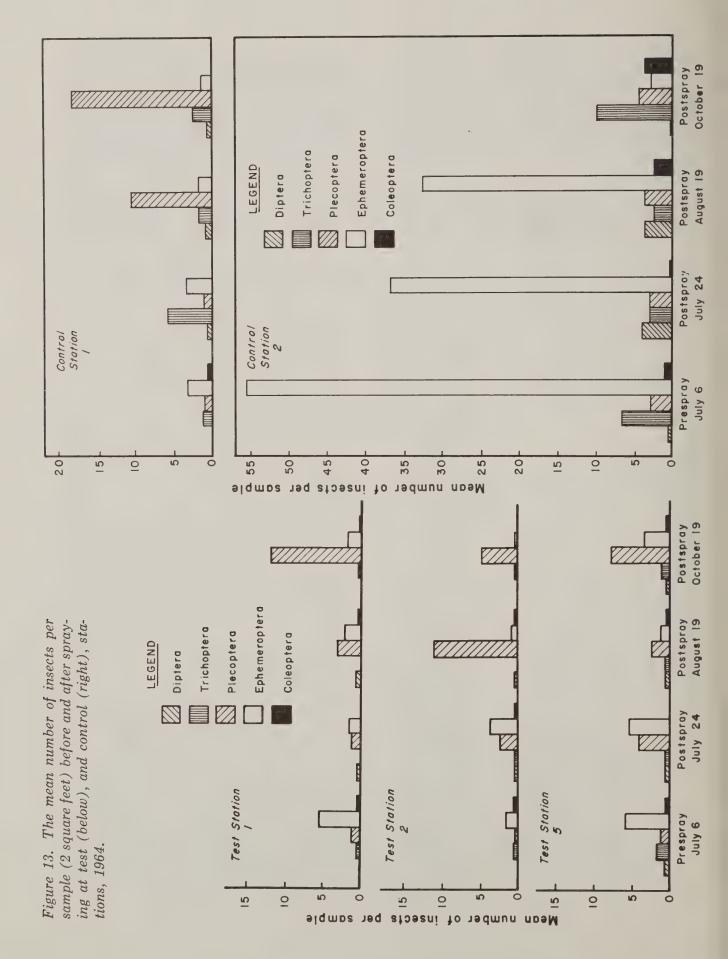
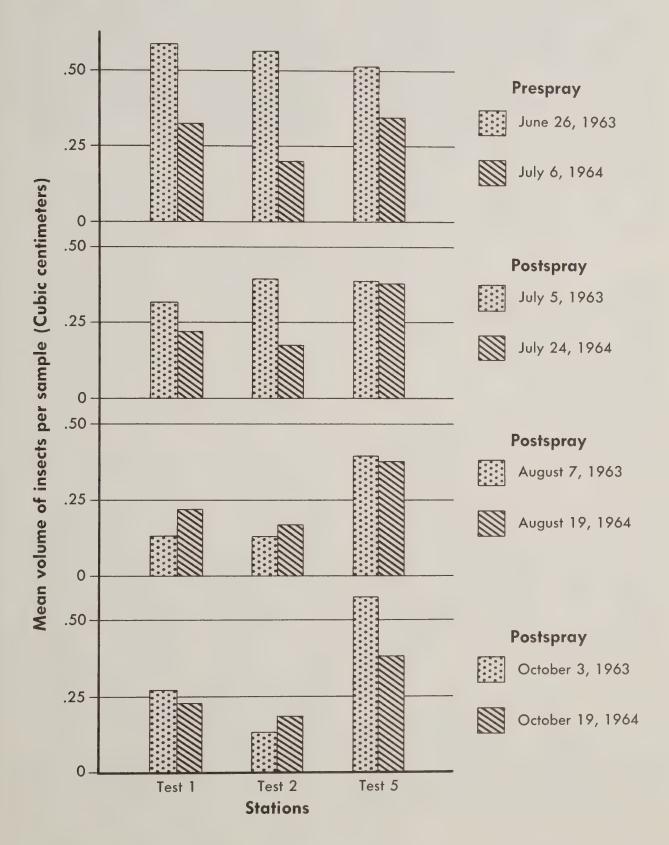


Figure 14. The mean volume of insects per bottom sample collected in 1963, compared with the same station and sampling period for 1964. The comparisons are made between the same stations, even though the station numbers were changed in 1964. The 1964 station numbers are used in this figure.



more pronounced than the reduction in 1964. In 1964, however, the prespray population was substantially smaller than in 1963.

In 1963, the predominant aquatic insect species were mayflies (fig. 12). They averaged approximately 10 per prespray sample. In 1964, mayflies also were predominant (fig. 13), but they averaged only four insects per sample. This reduction could have been due to annual environmental variation or some

other natural factor, rather than the presence of DDT.

In 1963, the mayfly population decreased substantially but rebounded significantly at three of the four test stations. In contrast, the 1964 mayfly population started out at a low level, decreased, presumably by the spraying activity, and never did rebound to its original prespray magnitude in any of the test station sampling areas, except the first postspray sample at station T-5.

SAMPLING OF DRIFTING INSECTS

1963

In addition to bottom insect samples, samples of drifting insects were collected with drift nets before and during spraying. The drift nets had a 1-square-foot opening with the net made of aluminum screen (14 meshes per inch). Drift net samples were taken for 15 minutes on an hourly schedule at most stations.

There were few insects found drifting in the streams when the prespray samples were taken on June 28 (fig. 15).

On June 30, the day of the 0.5 pound spraying by helicopter, increased numbers of insects were taken in the drift nets at test stations 2, 3, and 7, but not at stations 1, 4, and 6. It is obvious from these data that some spray did enter Hughes Creek. Test stations 1, 4, and 6, were located in the North Fork of the Salmon River below the mouth of Hughes Creek, in Ditch Creek, and in the West Fork of Hughes Creek, respectively.

Increased numbers of insects, many of them dead or moribund, were taken in the drift nets at test station 4, beginning on the second day of spraying. Ditch Creek was not one of the protected streams and no attempt was made to avoid spraying over the stream with the TBM-type aircraft. The large numbers of insects taken in the drift nets at this station corresponds with the decreased number of bottom organisms found in the bottom samples and in fish mortalities in the live boxes.

1964

Samples of drifting insects were collected in 1964 in the same manner and with similar equipment as that used in 1963.

In the prespray samples of July 8, small numbers (approximately five insects per sample) of insects were found to be drifting in the stream (fig. 16).

Moderate to large increases (from 12 to 100 insects per sample) in drifting insects were detected at each test station that was operated on both days of helicopter spraying. Test stations T-1, T-2, T-3, and T-4 were not operated on July 10, since the helicopters were spraying only in the vicinity of T-5.

During the TBM spraying of July 11, increases in drifting insects were even more pronounced. At test station 3, the number of drifting insects increased 11,000 percent more than the prespray average of July 8. At test station 5, the increase was 3,600 percent more than the prespray average.

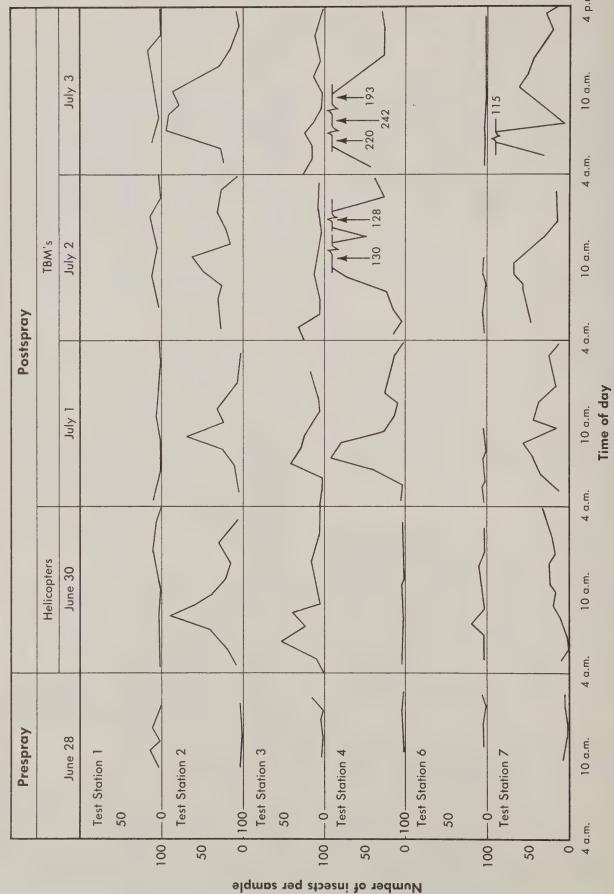
A short term experiment, conducted in 1964 by the authors, indicated that the presence of DDT is difficult to detect, by using the number of drifting aquatic insects as an indicator, if the DDT is applied to the stream more than 1½ miles from the drift insect sampling station. In this experiment, it was noted that only a 315 percent increase in drifting insects was observed when DDT was added to a stream (flow of 4 cfs) to make a 0.4 ppm concentration (to simulate 1 pound per acre DDT) a distance of 1.5 miles above the sampling site.

On July 16 and 17, the TBM sprayed the forested areas above 6,000 feet in elevation. This was more than 1½ miles above all of our drift sampling stations except station 5 and possibly station 4. These two stations were the only areas that showed slight increases in drifting insects on July 16. No increases were observed on July 17, when the upper extremities of the Ditch Creek area were sprayed.

1963 and 1964

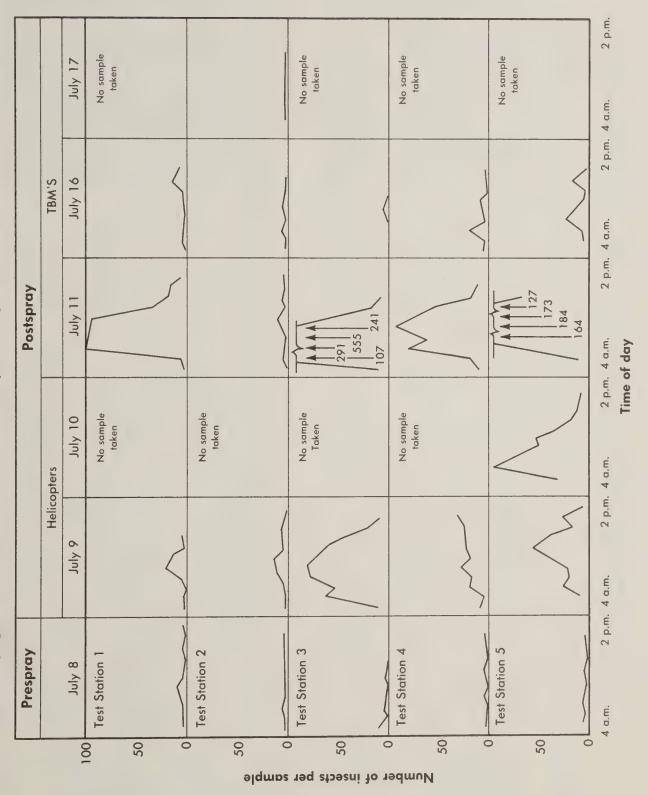
By reviewing the drift insect and water sampling data for 1963 and 1964, it is apparent that some DDT was deposited in the stream on each day of spraying. During each year, much higher increases in drifting insects

Figure 15. The number of aquatic insects collected in 15-minute drift-net samples before and during spraying at test stations, 1963.



 ^{1}T -4 station was on an unprotected stream. T-7 station was just below the mouth of an unprotected stream.

Figure 16. The number of aquatic insects collected in 15-minute drift-net samples before and during spraying at test stations, 1964.



drift-net samples before and during spraying at test stations in the Hughes The number and percentage of various orders of aquatic insects collected in Creek test area, 1963 Table 11.

					Test	Test stations						
Insect	4		2		3		4	- E	5 .		9 .	
orders	Number	Number Percent	Number rercent	Percent	Number	Number Percent	Number	Number Percent	Number	Number Percent	Number Percent	Percent
Ephemeroptera	39	30	585	46	167	29	637	31	œ	17	312	27
Plecoptera	29	22	180	14	85	15	405	19	11	24	83	7
Trichoptera	45	35	287	23	213	38	572	27	٥	19	520	45
Diptera	13	10	145		62		119	9	19	40	89	ω
Coleoptera	က	က	75	9	40	7	359	17	0	0	147	13
									1	1		
Totals	129	100	1,272	100	267	100	2,092	100	47	100	1,151	100
Number of samples	32	2	47	7	53	m	5	55	n	32	44	4

The number and percentage of various orders of aquatic insects collected in drift-net samples before and during spraying at test stations in the Hughes Creek test area, 1964 Table 12.

					Test s	Test stations					
Insect	Number	1 Number Percent	Number Percent	Percent	Number Percent	Parcant	A Number Percent	Porcont	Nimbor Boreant	Dorog	
	70.4	7.5			1 0 7 7	7.7	170	1100101	To Control of the Con		
Epnemeroprerd	204	0/	47	70	1,200	- \	107	45	906	90	
Plecoptera	9	_	4	က	154	6	13	2	73	5	
Trichoptera	49	7	13	٥	76	4	78	13	411	26	
Diptera	7.5		85	58	229	13	201	35	200	12	
Coleoptera	31	5	14	10	61	က	31	2	17		
_										1	
Totals	999	100	145	100	1,786	100	584	100	1,607	100	
Number of samples	39		48	~	37		38	~	52	~	

were observed when the fixed-wing aircraft were spraying than when the helicopters were spraying.

The insect sampling program was designed to collect only the larger insect forms; therefore, some of the smaller insect forms, especially *Diptera* larvae, are not represented in their true abundance. A limited number of samples with a small mesh net indicated large numbers of smaller insects, mostly *Diptera*, drifted in the stream after spraying commenced.

The mayflies were abundant in the drift

net samples, but other groups were also numerous (tables 11 and 12).

At some stations, species representing the orders *Tricoptera* and *Diptera* were more abundant in the drift catches than the mayflies. The differences in abundance of the various orders of insects in the bottom and drift-net samples may have been because essentially only one habitat type was sampled in the bottom-sampling program or that the various orders were not equally affected by DDT.

NOCTURNAL SAMPLING OF DRIFTING INSECTS

1963

Nocturnal samples of drifting insects were not taken in the 1963 study.

1964

In an effort to establish an index of the aquatic insect population before and after spraying, drifting insect samples were taken during the night from 8 p.m. until 4 a.m. They were spaced at 1-hour intervals for a length of 15 minutes per sample and were collected with the same drift nets as those used in the other drift insect sampling (1-square-foot opening at mouth of net with 14-mesh-per-inch aluminum window screen for the net material).

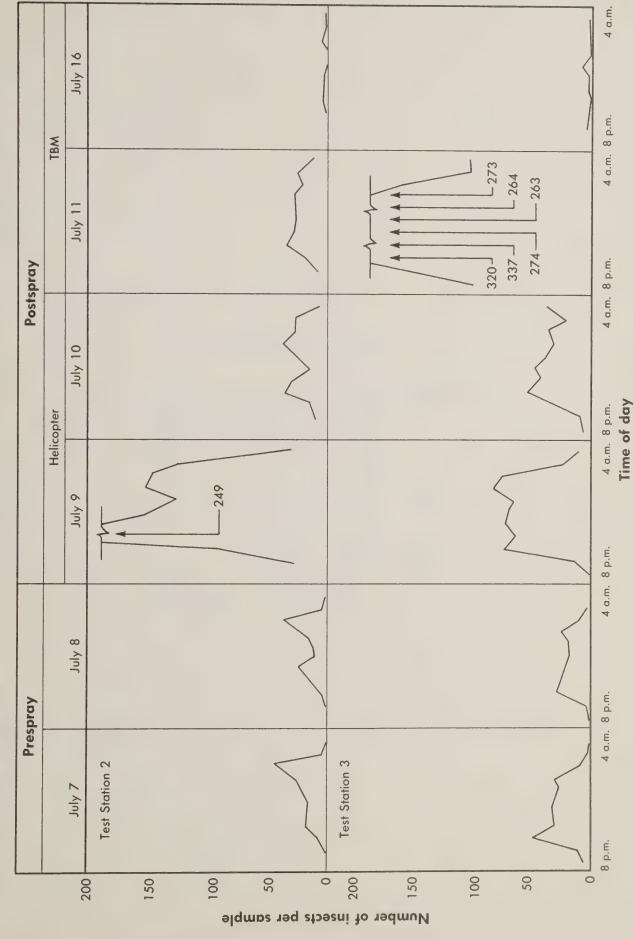
Other experimenters (R. Graham, by personal contact, 1964) have found that the number of drifting organisms in a normal stream will begin to increase in the evening hours, reach a peak during the night, and then

decrease as the sun rises in early morning. This same pattern of drift was found in Hughes Creek in the prespray samples of July 7 and 8 (fig. 17).

When spraying commenced, the number of nocturnally drifting insects collected in each drift sample was noticeably higher than in the prespray samples. Again, the fixed-wing aircraft were responsible for the largest increases in drifting insects. There was a 1,585 percent increase over the prespray average at station 3 on July 11.

On July 16, at both test stations, the mean number of drifting insects per sample was only about 25 percent of the mean number of insects per sample in the prespray samples. If the number of drifting insects is a true indication of the size of the aquatic insect population in the stream, then we have some indication that the insect populations above these stations were reduced substantially by the spraying activity, possibly by as much as 75 percent.

Figure 17. The number of aquatic insects collected in 15-minute drift-net samples between 8 p.m. and 4 a.m. before and during spraying at two test stations, 1964.



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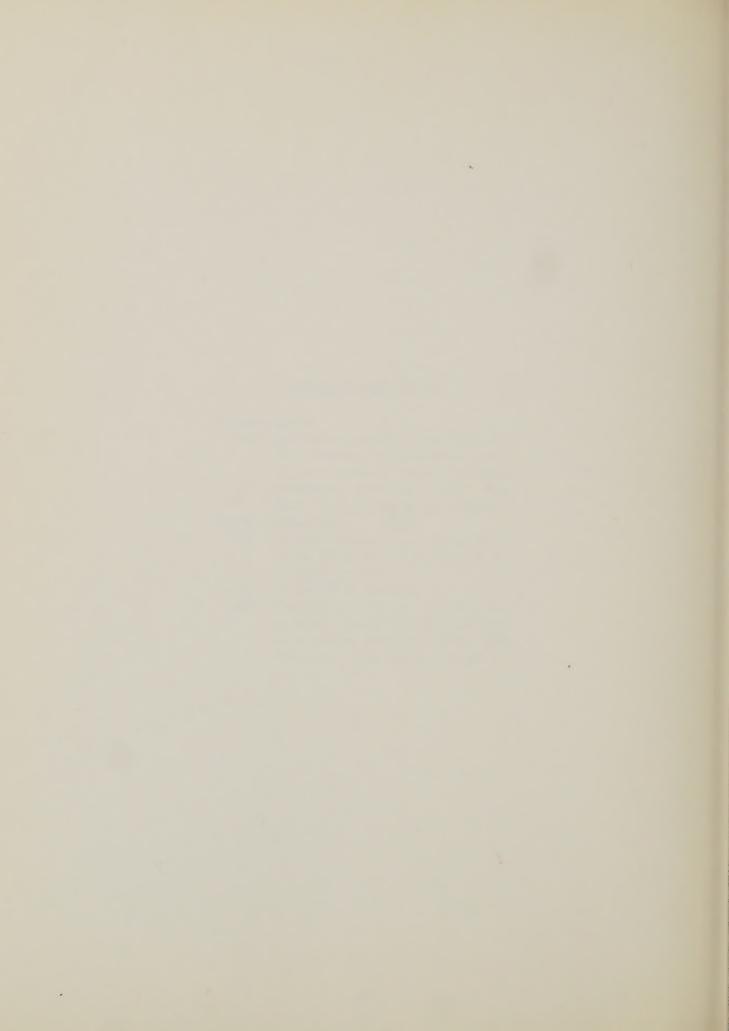
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